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Microbiological Characteristics of Urban Storm Water Runoffs in Central Ontario

Research Report No. 87

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arch Program for the Abatement of Municipal Pollution under Provisions of the Canada-Ontario Agreement on Great Lakes Water Quality

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MICROBIOLOGICAL CHARACTERISTICS OF URBAN STORM WATER RUNOFFS IN CENTRAL ONTARIO

RESEARCH PROGRAM FOR THE ABATEMENT
OF MUNICIPAL POLLUTION UNDER THE
PROVISIONS OF THE CANADA-ONTARIO
AGREEMENT ON GREAT LAKES WATER QUALITY

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MICROBIOLOGICAL CHARACTERISTICS OF STORM WATER RUNOFFS AT EAST YORK (TORONTO) AND GUELPH SEPARATE STORM SEWERS - Project No. 74-8-25
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Ontario Ministry of Environment

MONITORING OF STORM WATER RUNOFFS FOR BACTERIAL AND VIRAL PATHOGENS OF MAN - Project No. 76-8-41
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FOREWORD

On August 13, 1971, the Government of Canada and the Government of the Province of Ontario signed an Agreement (referred to as Canada-Ontario Agreement) to ensure that the water quality of the Great Lakes is restored, preserved and protected. Under the Canada-Ontario Agreement (COA), a number of research projects have been funded to achieve the specific water quality objectives set out in the agreement. The Urban Drainage Subcommittee (UDS) was appointed to examine and monitor, through various programs, water quantity and quality at a number of urban sites in Ontario, Canada.

As a part of UDS programs, three "feasibility" studies were designed to collect baseline data and information on the microbiological quality of urban storm water runoff by examining several storm sewers in Southern Ontario.

The first study (COA Project 75-3-24) was carried out by the Microbiological Laboratories of the Canada Centre for Inland Waters (CCIW). During the six months (June to November 1975) study period, the microbiological composition of storm water runoffs was examined in three areas having different land uses and drainage characteristics. These included one commercial area, Aldershot Plaza in Burlington, and two residential areas, Malvern Road, Burlington, and Brucewood subdivision, Borough of North York in Metropolitan Toronto. Samples, collected at varying times during several storm events, were analyzed for pollution indicator bacteria (total coliforms, fecal coliforms, fecal streptococci), pathogenic bacteria (*Pseudomonas aeruginosa*, salmonellae), total fungi, parasites and coprostanol.

The second study (COA Project 74-8-25) was conducted by the Microbiological Laboratories of the Ontario Ministry of the Environment during September 1975 to February 1976. In this investigation, the incidence and distribution of pollution indicator bacteria, pathogenic bacteria and total fungi were examined in storm water runoff samples collected every five minutes for a period of one hour during the course of a rainfall event. The source(s) of microbiological contamination and potential health hazards associated with storm water pollution were also

evaluated. Several storm events were monitored at two sites with different topography and drainage characteristics. The sites studied were: a residential area along Barrington Avenue in the Borough of East York in Metropolitan Toronto, and a residential-commercial area in the vicinity of Woodlawn Road in Guelph.

The third study (COA Project 76-8-41) was also undertaken by CCIW Microbiological Laboratories during April to November 1976. This investigation specifically examined the occurrence and time-related distribution of pathogenic bacteria in storm water runoff samples collected at different time intervals over the first four-hour period of a rainfall event. In addition, the incidence of pollution indicator bacteria, heterotrophic bacteria, total fungi, Leptospira and viruses was determined in these samples. A storm sewer outfall in the residential area of Malvern Road in Burlington was the site of this study.

Information regarding the sites examined, methods and techniques used, and the results and findings of these studies were documented in three separate reports. Although these investigations were conducted in diverse areas using three different approaches, they essentially dealt with a common subject. Therefore, the three reports are compiled here to bring all data and comprehensive information on the microbiological characteristics of Canadian storm water runoffs under one cover. Some of the highlights and conclusions of these studies are summarized below.

1) High densities of pollution indicator bacteria (total coliform, fecal coliform and fecal streptococcus) were found in the storm water runoff samples collected from all five separate storm sewer systems examined.
2) High concentrations of heterotrophic bacteria and fungi, indicative of excessive organic enrichments, were present in all storm water runoffs.
3) Among the pathogenic micoorganisms tested for, Pseudomonas aeruginosa were the most numerous and consistently recovered. Salmonellae were detected quite frequently both in the individual and composite storm water samples. Staphylococcus aureus were infrequently isolated and only in low numbers. No human pathogenic Leptospira were isolated. Only one sample, collected from the latter part of a storm event, was positive for human pathogenic virus (reovirus).

4) Infiltration waters appeared to be a source of low level but continuous microbial pollution. Similarly sediments, which not only contained significant quantities of indicator organisms but pathogens as well, constitute a reservoir for various microorganisms that ultimately find their way into storm water runoffs.

5) The levels of microbial populations in storm water runoffs were strikingly high and were similar to those encountered in dilute sewage, and therefore constitute a health hazard. The implied public health risks are substantiated by the consistent recovery of pathogenic microorganisms in discharges at the sites studied.

6) The results of these studies indicate that fecal pollution in the separate storm systems examined is predominantly of non-human origin. Fecal material from animals and birds washed into the systems during rain storms appears to be the major source of microbial pollution.

7) There appeared to be little relationship between the intensity and amount of rainfall and the occurrence of indicator and pathogenic microorganisms. As a result, no typical patterns of distribution of these organisms in storm water runoffs could be established in these studies. The peak microbial populations were found either at the initial stages, middle or the tail-end of the sampling periods of different storm events monitored. Pathogenic bacteria were detected during all phases of the 240-minute sampling periods in one study. The unpredictable distribution patterns and intermittent release of microorganisms in storm waters suggest that any effect of "initial flushing" on microbiological quality of storm water runoffs was minimal during an individual storm event. Therefore, to remove the health hazard and to improve the quality of discharges, the collection and subsequent treatment of a specific portion of each storm would be of little significance. This does not necessarily mean, however, that for removing and preventing health hazards, disinfection of all urban storm water runoff would be required. A more realistic and practical solution would be to treat all sanitary sewage overflows and discharges from those separate storm sewers

where land uses and drainage characteristics (e.g. cross-contamination from domestic sewage) indicate the necessity for undertaking such an action.

8) These feasibility studies provide baseline data and significant information on the microbiological quality of storm water runoffs at several locations in central Ontario. In future, studies should be carried out to determine the nature and degree of microbiological loadings in receiving waters. Also, demonstration projects should be conducted to ascertain the most effective and economical remedial measures to prevent public health risks and deterioration of microbiological quality of surface waters receiving urban storm water runoff.

AVANT-PROPOS

Le 13 août 1971, le gouvernement du Canada et celui de la province de l'Ontario ont signé l'Accord Canada-Ontario qui vis à rétablir, conserver et protéger la qualité de l'eau des Grands lacs. Afin d'atteindre les objectifs de qualité de l'eau fixés par cet accord (ACO), on a financé certains projets de recherche. Le sous-comité de drainage urbain (SDU) a été formé afin d'examiner et de vérifier, par divers programmes, l'aspect quantitatif et qualitatif de l'eau dans certaines zones urbaines de l'Ontario.

Les programmes du SDU comprenaient, entre autres, trois études destinées à recueillir des renseignements et des données de base sur la microbiologie des eaux de ruissellement de certains égouts pluviaux dans le sud de l'Ontario.

Ce sont les laboratoires de microbiologie du Centre canadien des eaux intérieures (CCEI) qui ont mené la première de ces études (projet ACO 75-3-24). Durant les six mois qu'elle duré (de juin à novembre 1975), les techniciens ont examiné la composition microbiologique des eaux de ruissellement de trois zones où différaient les caractéristiques de drainage et l'utilisation des terres. C'est ainsi qu'ils ont choisi une zone commerciale, Aldershot Plaza à Burlington, et deux zones domiciliaires, Malvern Road à Burlington et un secteur de Brucewood, qui est un quartier de North York dans le centre de Toronto. L'analyse des échantillons, prélevés à divers moments au cours de plusieurs orages, a porté sur les bactéries indicatrices de pollution (nombre total de coliformes, coliformes et streptocoques d'origine fécale), sur les bactéries pathogènes (Pseudomonas aeruginosa, salmonellae), sur le nombre total de champignons, sur les parasites et sur le coprostanol.

La deuxième étude (projet ACO 74-8-25) a été menée par les laboratoires de microbiologie du ministère de l'Environnement de l'Ontario, de septembre 1975 à février 1976. Au cours de cette étude, l'examen des échantillons d'eaux de ruissellement, prélevés toutes les cinq minutes pendant une heure au cours d'une chute de pluie, a eu pour objet de déceler la présence et d'établir la répartition des bactéries indicatrices de pollution, des bactéries pathogènes et de l'ensemble des

champignons. On a aussi évalué le ou les sources de contamination microbienne ainsi que les risques sanitaires dus à la pollution par les eaux pluviales. L'étude, qui a porté sur plusieurs orages, s'est faite à deux endroits différant du point de vue du relief et des caractéristiques de drainage: une zone domiciliaire, le long de l'avenue Barrington, dans le quartier de York Est (centre de Toronto) et une zone, à la fois domicilaire et commerciale, avoisinant Woodlawn Road, à Guelph.

La troisième étude (projet ACO 76-8-41), aussi entreprise par les laboratoires de microbiologie du CCEI, a duré d'avril à novembre 1976. Cette étude s'est attachée plus particulièrement à déceler la présence de bactéries pathogènes et à établir leur répartition temporelle dans des échantillons d'eaux de ruissellement, prélevés à divers moments au cours des quatre premières heures d'une chute de pluie. On y a en outre recherché la présence de bactéries indicatrices de pollution, de bactéries hétérotrophes, de l'ensemble des champignons, de la Leptospira et de virus. Les prélèvements provenaient d'un émissaire pluvial de la zone domiciliaire de Malvern Road, à Burlington.

Les renseignements sur les lieux examinés, les méthodes et les techniques employées, les résultats et les conclusions de ces études se trouvent consignés dans trois rapports distincts. Bien que ces études aient été menées dans diverses zones et que la méthode ait différé chaque fois, elles portent essentiellement sur le même sujet. Les trois rapports ont donc été réunis de façon à grouper toutes les données et à fournir une information exhaustive sur les caractéristiques microbiologiques des eaux de ruissellement au Canada. Quelques faits saillants et certaines conclusions sont résumés ci-après:

1) On a trouvé, dans les échantillons d'eaux de ruissellement prélevés dans les cinq réseaux d'égout séparatifs examinés, une forte concentration de bactéries indicatrices de pollution (nombre total de coliformes, coliformes et streptocoques d'origine fécale);
2) Dans tous les écoulements pluviaux, on a décelé une concentration élevée de bactéries hétérotrophes et de champignons, signe d'une abondance excessive de matières organiques;
3) Parmi les micro-organismes pathogènes recherchés, c'était la Pseudomonas aeruginosa qui était en plus grand nombre et constamment

présente. On a trouvé assez souvent des salmonelles dans les échantillons d'eau de pluie soit isolés, soit mélangés. Le Staphylococcus aureus n'est apparu que quelques fois et en petit nombre. On n'a décelé aucune Leptospira, organisme pathogène pour l'homme. Un seul échantillon, prélevé vers la fin d'un orage, a révélé la présence d'un virus pathogène pour l'homme (un réovirus);

4) Les eaux d'infiltration semblent être une source faible mais continue de pollution microbienne. De la même façon, les sédiments, dans lesquels on trouve non seulement des quantités importantes d'organismes indicateurs, mais aussi des microbes, constituent un réservoir de nombreuses sortes de micro-organismes que les eaux pluviales finissent par entraîner;

5) La concentration de microbes dans les eaux de ruissellement, qui a été incroyablement élevée, se compare à celle des eaux d'égout diluées; elle représente, par conséquent, un danger pour la santé. Ces risques se sont confirmés par la présence constante de micro-organismes pathogènes aux émissaires des lieux étudiés;

6) D'après les résultats de ces études, la pollution d'origine fécale dans les réseaux d'égout séparatifs ne met guère l'homme en cause. Il semble plutôt que la principale source de pollution microbienne provient des matières fécales d'animaux et d'oiseaux, entraînées dans ces réseaux par la pluie;

7) Il a semblé y avoir peu de relation entre l'intensité et la quantité des précipitations, d'une part, et la présence de micro-organismes indicateurs de pollution et pathogènes, d'autre part. En conséquence, il a été impossible, au cours de ces études, de dégager un modèle typique de répartition de ces organismes dans les eaux de ruissellement. Durant les orages étudiés, la concentration maximale de microbes pouvait se situer tantôt au début, tantôt au milieu, tantôt à la fin de l'échantillonnage. Au cours d'une de ces études, on a pu déceler des bactéries pathogènes dans tous les prélèvements effectués dans l'espace de 240 minutes. La répartition imprévisible des micro-organismes et leur libération irrégulière dans les eaux pluviales laissent supposer que la flore microbienne présente n'est guère modifiée, au cours d'un orage, par le lessivage initial. Cela

signifie que la retenue et le traitement subséquent d'une partie quelconque des eaux de chaque orage ne contribueraient guère à éliminer le danger et à améliorer la qualité des eaux évacuées. Il n'y a pourtant pas lieu de conclure qu'on doive absolument désinfecter toutes les eaux pluviales urbaines pour éviter les risques de contamination. Une solution plus réaliste et plus pratique serait de traiter toutes les eaux-vannes qui débordent et qui s'écoulent des réseaux d'égout séparatifs là où les caracéristiques de drainage et d'utilisation des terres nécessitent une intervention (c'est-à-dire quand les eaux domestiques contaminent les pluviales);

8) Ces études ont fourni d'importants renseignements et des données de base sur le genre de micro-organismes présents dans les eaux de ruissellement de plusieurs points du centre de l'Ontario. Les prochaines études devraient porter sur la nature et l'importance des micro-organismes dans les eaux réceptrices. On devrait aussi entreprendre des projets témoins pour déterminer les mesures correctives les plus efficaces et les plus économiques qui permettent de parer aux menaces à la santé publique et à la détérioration des propriétés microbiologiques des eaux de surface qui reçoivent les eaux de ruissellement.

MICROBIOLOGICAL STUDY OF SOME CANADIAN STORM WATER RUNOFFS AT BURLINGTON AND BRUCEWOOD

by

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Project No. 75-3-24

Bacteriological Data Collection
for the
Urban Drainage Subcommittee
Canada-Ontario Agreement on Great Lakes Water Quality

ABSTRACT

A six-month study (June-November, 1975) was undertaken to investigate the microbiological composition of storm water runoffs in Burlington, Ontario, and Brucewood (Toronto). Samples were collected at varying times throughout several storm events. The samples were analyzed for coliforms, fecal coliforms, fecal streptococci, *Pseudomonas aeruginosa*, fungi, salmonellae, ova and parasites, and coprostanol. Background data were obtained from infiltration samples and sediments.

Problems encountered with sample collection and the establishment of zero hour and peak density times are described.

RÉSUMÉ

Une étude menée de juin à novembre 1975 a eu pour objet d'examiner la composition microbiologique des eaux de ruissellement à Burlington et à Brucewood (Toronto), en Ontario. Le prélèvement des échantillons s'est fait à divers moments au cours de plusieurs orages. On a procédé à leur analyse en vue d'y déceler les coliformes ainsi que les coliformes et streptocoques d'origine fécale, la Pseudomonas aeruginosa, les champignons, les oeufs et les parasites, ainsi que le coprostanol. On a dérivé des données de base de l'analyse des échantillons d'eau d'infiltration et de sédiments.

Le rapport décrit les problèmes que pose le choix de l'instant propice à commencer l'échantillonnage ou les périodes de concentration maximale des micro-organismes.

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CONCLUSIONS

1) Sample collection by manual and automatic means, as presently practiced, leaves much to be desired. Zero hour samples must be collected as soon as background water levels increase, possibly by as little as 1 cm.

2) Samples collected automatically should be collected in sterile refrigerated containers.

3) Manual sample collection should be carried out by someone who lives beside or near the sample collection site. Perhaps a local resident could be employed on a stand-by basis for the duration of a survey.

4) Coliforms in the infiltration samples can reach as high as 10^7 coliforms per 100 ml, causing one to suspect regrowth problems.

5) Storm water microbial levels are similar to dilute sewage, contain organisms recognized as health hazards, and reach peak populations within 45 minutes of storm initiation.

6) Pathogenic microorganisms are believed to move in slugs. Therefore, it may be more appropriate to sample more frequently and to test larger quantities of water for pathogens to establish an indication of the risks involved.

7) The following microorganisms are believed to have adequate isolation procedures and should be included in a pathogen sampling program: salmonellae, Pseudomonas aeruginosa, viruses, human and animal invasive fungi, and two relatively obscure pathogens which are routinely tested for in Europe, Vibrio parahaemolyticus and Leptospira.

1. INTRODUCTION

Over the past decade there has been an increasing awareness of the heavy pollutional load on receiving waters from urban storm water runoff. Some of the sources of this pollution are debris and contaminants from streets, soil-chemical mixtures from open land areas, garden chemicals, airborne wastes, engine oil and combustion products, ice control chemicals and fecal material from animals and birds [1,2,3,4,5,6,7].

Geldreich et al [1] reported that storm waters from city streets, suburbs and wooded hillsides had bacterial densities similar to storm water runoff from cultivated farm land. Other studies [1,3,5,6] have shown that runoff from street surfaces is highly contaminated, bacteriologically. Sartor et al [6] reported that urban storm runoff is similar in many respects to sanitary wastewater. Calculations based on a typical city indicate that the runoff from the first hour of a moderate to heavy storm (1.27 cm/hr) would contribute a considerably greater pollutional load than would the same city's sanitary waste during the same time period.

Studies on the sources of the bacteria found in storm water runoff from residential and light commercial areas indicated that the bacteria were predominantly of non-human origin [1,3]. However, in several instances [1,2,5], pathogenic bacteria were isolated in runoff waters, demonstrating the existence of a potential health hazard.

Very little data exists on the microbiological composition of Canadian storm water runoffs. This study was undertaken to fill this gap, and concentrate on the central Ontario region of Canada. Microbiological data from several storms during the June 1975 to November 1975 period are presented in this report. Samples were collected from three different locations, one commercial and two residential.

2. STUDY SITES

2.1 Aldershot Plaza, Burlington

The Aldershot catchment area comprises 17 acres of commercial land in the western section of Burlington. The imperviousness of the catchment is 100%, with roofs, sidewalks and paved parking lots making up all of the area. The plaza contains one large department store, a Canadian Tire workshop and gas pump, one large chain grocery store and 15-20 small stores. The area is serviced by separate sewers.

2.2 Malvern Road, Burlington

The Malvern Road area is a 57.6-acre section in central Burlington. The entire area is zoned as single-family residential and there are no vacant lots within the area. Most of the houses were built in the early 1960's with virtually all development completed by 1964. The sewers are separated.

2.3 Brucewood Area, Toronto

The Brucewood subdivision is a 48.23-acre development located in the northeast corner of the Borough of North York in Metropolitan Toronto, about 15 miles from the centre of the city. It consists entirely of large single-family detached and semi-detached residences built in the late 1960's. There are 169 detached units and 43 semi-detached, and roof leaders are connected to separated storm sewers. The total imperviousness of the catchment is 48.3% with streets accounting for 7.0 acres, driveways 4.61 acres and roofs 11.69 acres.

3. MICROBIOLOGICAL SAMPLE COLLECTION PROCEDURES

3.1 Aldershot Plaza

Microbiological and coprostanol samples were collected by means of a North Hants automatic sampler. This sampler was triggered by a 5 cm rise in water level within the storm sewer. Sampler and weir were constructed within a manhole structure (Figure 1).

Up to twenty-four 900-ml samples could be collected by the North Hants sampler at 5-15 minute intervals through individual tubes.

If the storm occurred late in the day or during the night, samples would remain in their containers until the technician removed them the following morning. Because of the delay and resultant microbial action, microbiological and chemical data must be suspect. Samples were usually processed within 18 hours of collection.

During one storm, microbiological samples were collected by hand as well as by the automatic sampler, for comparison purposes. Samples were iced and returned to the laboratory for processing.

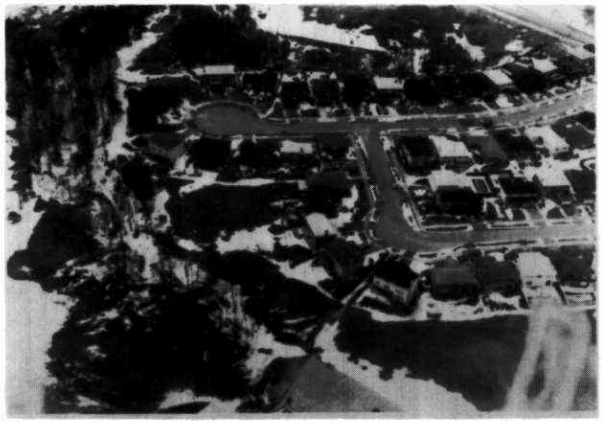
No sediment samples were collected as flow rates were sufficiently high to prevent the build-up of any sediment (Figure 1).

Sample collection problems similar to those occurring at Malvern, and described below, also occurred. In mid-August a heavy storm completely demolished and swept away all sampling equipment, thus ending our sampling program at this site.

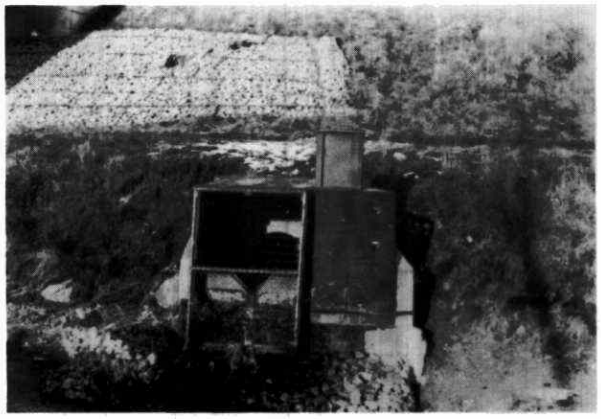
3.2 Malvern Road

Microbiological and coprostanol samples were usually collected by means of a SIVCO sampler B/ST-VS/2YA-EX in one-litre aliquots. The sampling cycle consisted of a pre-sampling purge, sample withdrawal and a post sampling purge. The sampler was activated automatically when the level of storm water in the sewer outfall (Figure 1) rose approximately 4 cm. Sampler operation was terminated in two ways: either after 24 samples had been collected at 5, 15 or 30 minute intervals, or after the water level fell below 4 cm.

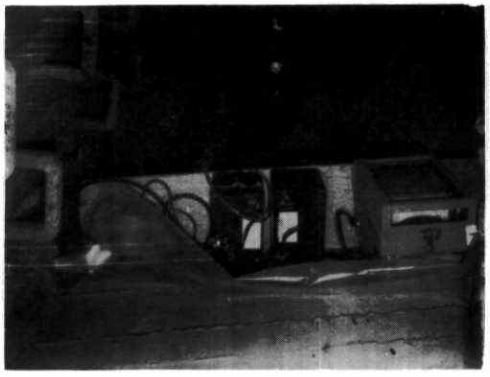
If the storm occurred late in the day or during the night, samples would remain in their containers until the technician removed them the following morning. Because of the delay and resultant microbial



Grand view of typical, partially wooded area of Brucewood catchment area.



Brucewood outfall, showing sediment trap. Microbiological samples hand-collected here



Manhole in Aldershot showing automatic vacuum sampler, which collected microbiological samples. Catchment area is 100% commercial and paved.

Aldershot stormsewer showing lack of sediment build up due to heavy water flows.

Malvern outfall on creek within urban Burlington. No vacant lots within catchment area. Microbiological samples collected by Sivco Sampler.

FIGURE 1. GENERAL VIEW OF SAMPLERS AND SAMPLING SITES BRUCEWOOD, ALDERSHOT AND MALVERN

action, microbiological and chemical data must be suspect. Samples were usually processed within 18 hours of collection.

During one storm, microbiological samples were collected by hand as well as by the automatic sampler, for comparison purposes. Samples were iced, returned to the laboratory and processed.

Sediment samples were collected from within the weir (Figure 1) by hand, using a sterile spatula. Approximately 100 grams of surface sediment was collected and placed in a sterile "Whirl Pak" bag and stored on ice in an insulated chest. Sediment samples were processed within 18 hours.

Many problems arose with the automatic sampler collections. For a variety of mechanical reasons, many samples could not be collected because the sampler did not trigger. In several instances a series of short storms occurred which made it difficult to follow a typical storm pattern. At other times, a long, light rain would not trigger the mechanism because the water level never reached the 4 cm level.

3.3 Brucewood

Bacteriological and coprostanol samples were hand collected at the weir outfall (Figure 1). At the first indication of rainfall, a technician would drive to the sampling site and collect microbiological samples at 10-15 minute intervals in sterile glass 500-ml bottles, and coprostanol samples in sterile two-litre plastic containers containing 1 ml 10% mercuric chloride as preservative. After collection, the samples were placed on ice in an insulated chest, returned to the laboratory and processed within 18 hours of collection.

Sediment samples were collected from within the weir by hand, using a sterile spatula. Approximately 100 grams of surface sediment was collected and placed in a sterile "Whirl-Pak" bag, and stored on ice in the insulated chest. Sediment samples were processed within 18 hours.

3.4 Infiltration Samples

At each sampling site, during periods of no rain, infiltration samples were hand collected from the regular collecting sites. Samples were collected in the morning, iced, returned to the laboratory and tested within four hours of collection. These samples provided baseline levels for all storm events.

3.5 Salmonella Sample Collection

Salmonella collection pads [8] were suspended in the storm water during the duration of each event. After the storm, the five "fingers" from each pad were cut free from their frame, placed in sterile "Whirl-Pak" bags, iced and returned to the laboratory for processing.

4. MICROBIOLOGICAL METHODS

Total coliform, fecal coliform, fecal streptococcus and Pseudomonas aeruginosa density estimates were performed on all samples, using the MF technique and media outlined in the Canada Centre for Inland Waters "Methods for Microbiological Analysis of Waters, Wastewaters and Sediments" [8].

During late July, fungal density estimates were initiated, as well as tests for the presence of ova and parasites [8].

On many storm water and infiltration samples, semi-quantitative salmonella tests were performed. One hundred-millitre and 50-ml aliquots of storm water were filtered through fibreglass prefilters and the filters placed in 100-ml aliquots of tetrathionate and selenite broths prewarmed to 41.5°C. Ten-millitre aliquots of storm water were also placed directly into 100-ml aliquots of tetrathionate and selenite broths. Cultivation and isolation procedures were followed as outlined in "Methods for Microbiological Analysis of Waters, Wastewaters and Sediments" [8]. Salmonella collection pads were treated as outlined in the above manual.

Sediment salmonella populations were estimated by placing 10-gram aliquots into three 300-ml volumes of tetrathionate broth and into two 300-ml volumes of selenite broths, and following routine salmonella culturing procedures [8].

Coliform isolates were collected from one infiltration sample at each sampling site and from one storm runoff at Brucewood as a means of typifying the normal enteric populations. Isolate identification procedures were performed as outlined by Dutka et al [9].

Samples for fecal sterol (coprostanol) level determination were analysed according to procedures described by Dutka et al [10].

5 RESULTS

5.1 Aldershot Plaza

Eight infiltration samples were hand collected for baseline data during the June 19 to September 9, 1975 period. Coliform isolates were collected and identified from the September 9 sample. Table 1 presents the microbiological data and the results of the coliform identification procedures.

TABLE 1. INFILTRATION SAMPLES, ALDERSHOT PLAZA STORM SEWER, BURLINGTON

Date	Total Coliforms /100 ml	Fecal Coliforms /100 ml	Fecal Strepto-cocci /100 ml	P. aeru-ginosa /100 ml	Fungi /100 ml
June 19	$>1.0 \times 10^5$	7.5×10^3	1.8×10^3	2.6×10^3	
June 23	$>1.0 \times 10^5$	4.1×10^3	1.2×10^2	2.6×10^3	
July 2	6.7×10^4	1.0×10^2	7.1×10^2	3.1×10^2	
July 7	5.6×10^6	2.8×10^2	5.1×10^3	1.7×10^3	
July 16	2.2×10^7	3.1×10^2	6.7×10^2	2.7×10^3	
July 29	9.0×10^3	3.1×10^2	2.1×10^2	1.4×10^1	2.0×10^6
Aug. 18	2.9×10^3	1.4×10^2	5.5×10^3	3.0×10^3	2.0×10^4
Sept. 9*	2.6×10^6	2.1×10^3	1.6×10^2	1.4×10^2	

*Coliform Isolate Identification (40)
50% - Aeromonas
45% - Klebsiella
5% - E. coli

From Table 1 and Figure 2 it can be seen that normal infiltration coliform populations varied between 10^3 to 10^7 and fecal coliform populations varied between 10^2 to 10^3 organisms per 100 ml. However, if the September 9 coliform isolate data were typical of all infiltration samples, then only 50% of these enumerated coliforms were enterobacteriaceae, and thus true coliforms. No salmonellae were isolated from these infiltration samples.

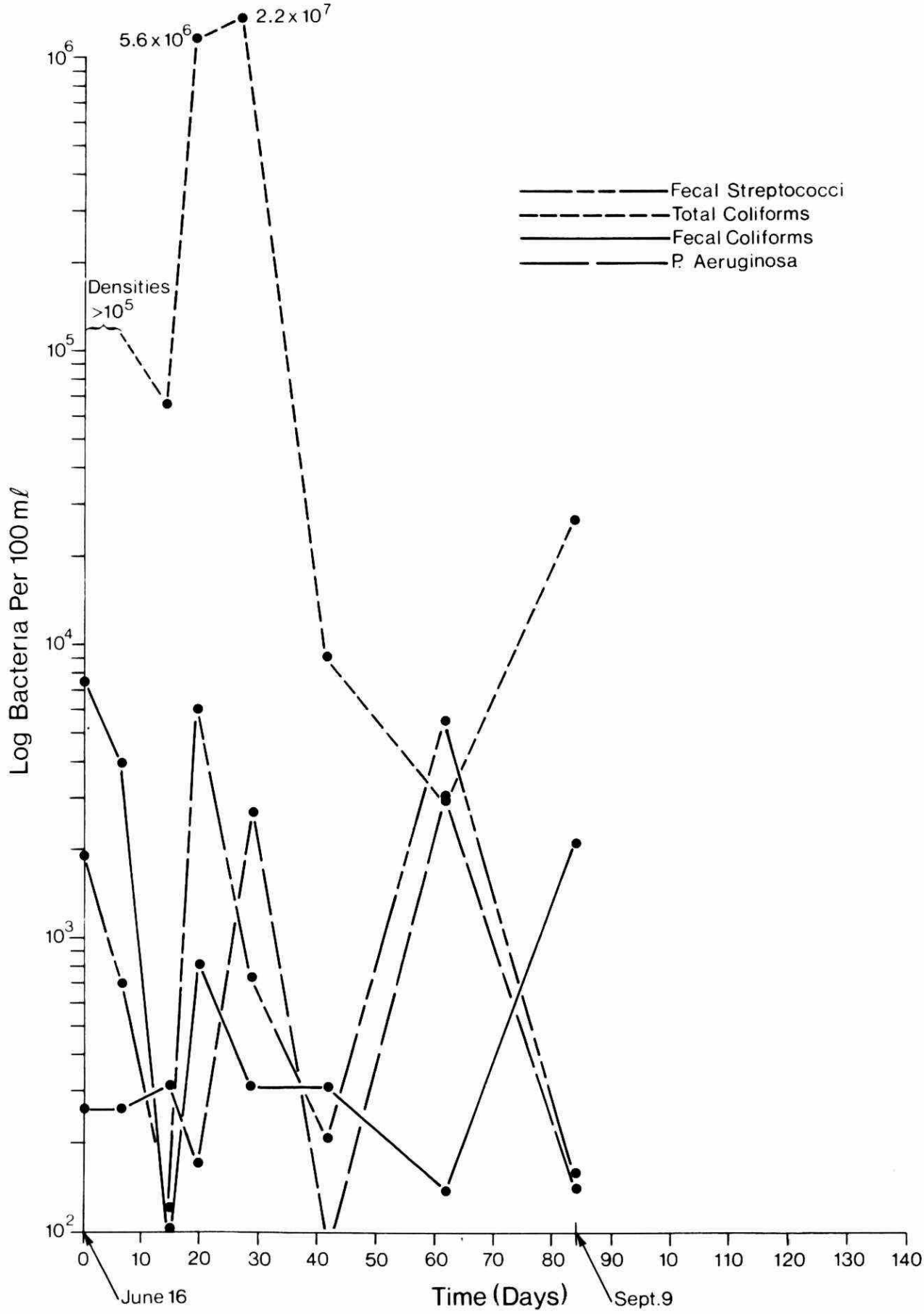


FIGURE 2. INFILTRATION DATA, ALDERSHOT PLAZA STORM SEWER, JUNE 16 - SEPTEMBER 9, 1975

Figure 2 data indicate fecal coliform and coliform populations peak in late June - early July and have minimum populations during the mid-August period. Pseudomonas aeruginosa and fecal streptococcus populations fluctuated widely during the sample collection period.

During one storm event, a comparison was made between normally collected samples and those collected by the automatic sampler. Results of this comparison are presented in Figure 3 and Table 2. It can be seen that sample collection procedures had little or no effect on bacterial populations. These data also indicate a very well-mixed storm water which probably moves along as a slug.

The results of all storm events from which microbiological samples were collected are presented in Table 2. In comparison to the infiltration data (Figure 2), it can be seen that all microbiological populations were greatly elevated during a storm, and were very similar to those found in dilute sewage. The storm of August 21 was the only storm runoff documented from beginning to end and here it can be seen that maximum populations occurred within the first hour, with different populations peaking at different times (Figure 4).

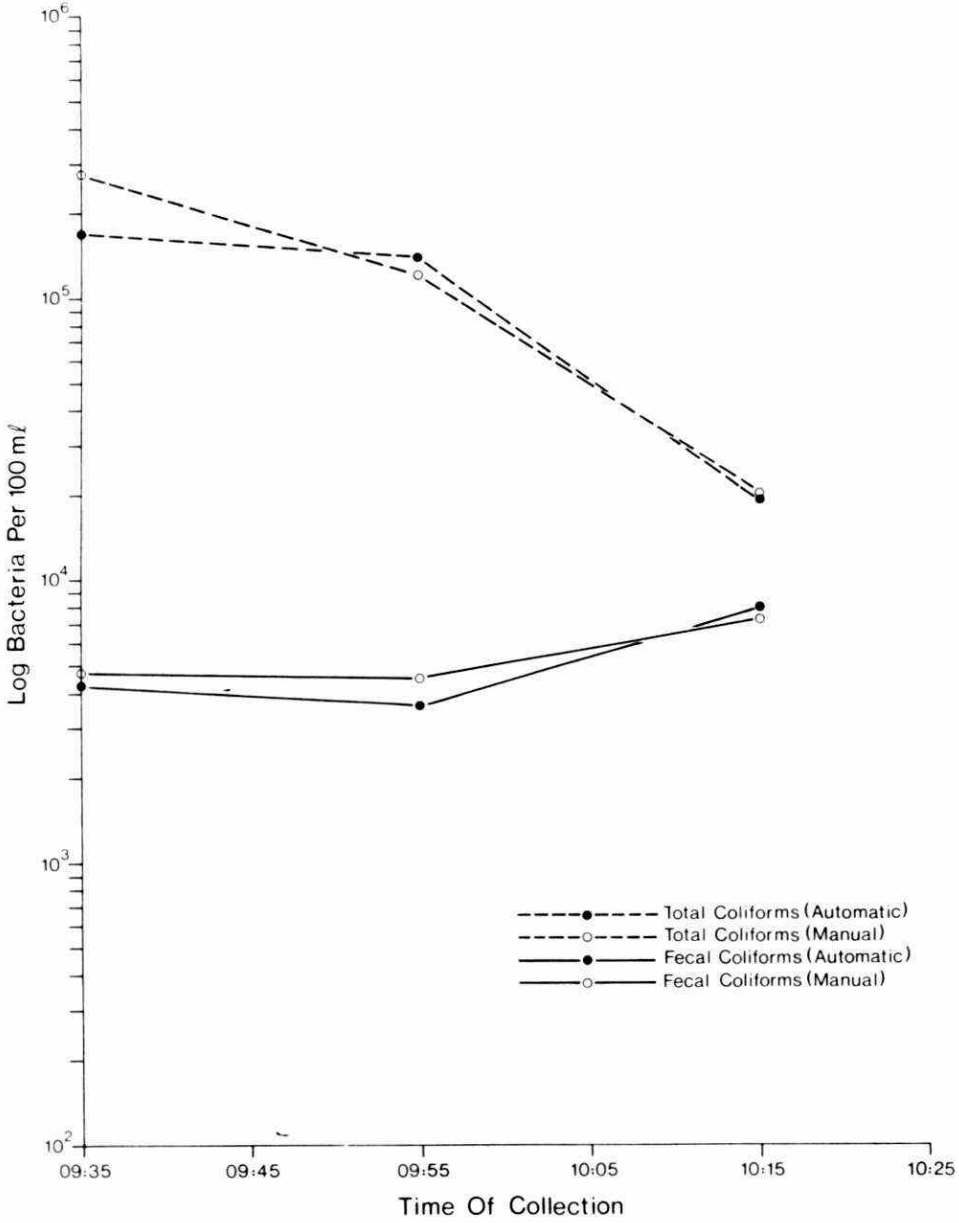
Salmonellae (S. senftenberg and S. newport) were isolated on two occasions by the collecting pad technique. Unfortunately, on both occasions the automatic sampler failed to operate and thus no microbiological background data were obtained.

Only on one occasion (August 11 event) were any parasites isolated. During this event a free-living nematode, probably belonging to the order Rhabditida, was found in one 100-ml sample.

5.2 Malvern Road

Eight infiltration samples were hand collected for base-line data during the period June 16 to September 9, 1975. Coliform isolates were collected and identified from the September 9 sample. In Table 3 the microbiological data and the results of the coliform isolate identification study are presented.

From Table 3 and Figure 5 it can be seen that microbial populations fluctuated between 10^1 to 10^3 organisms per 100 ml. Coliform



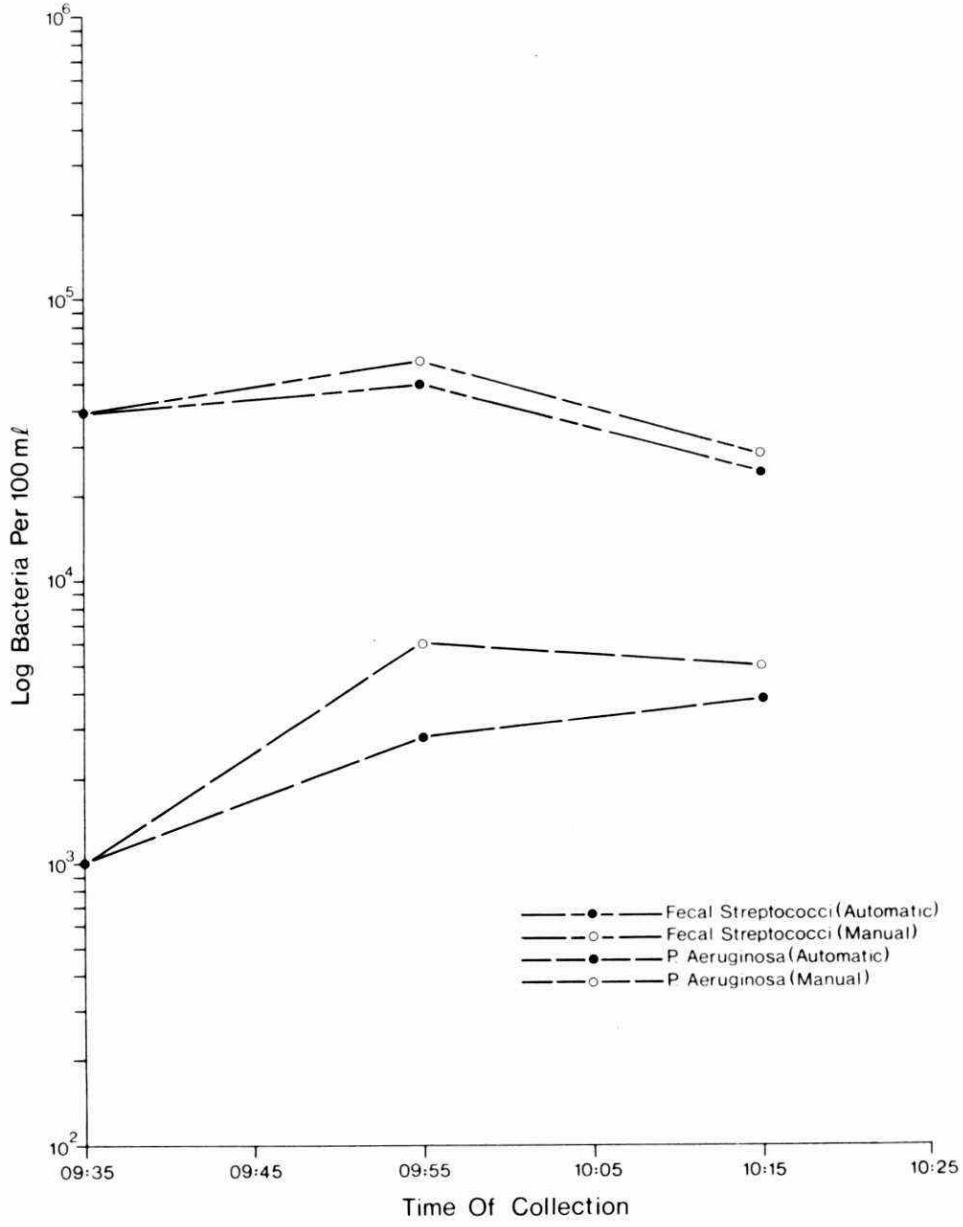


FIGURE 3. COMPARISON OF AUTOMATIC AND MANUAL SAMPLE COLLECTION PROCEDURES ON BACTERIAL POPULATIONS AT ALDERSHOT PLAZA STORM SEWER

TABLE 2. MICROBIOLOGICAL DATA FROM ALDERSHOT PLAZA STORM SEWER, BURLINGTON, JUNE 19 - AUGUST 21, 1975

Date	Storm		Method of Sample Collection	Time of Sample Collection	Coli-forms /100 ml	Fecal Coli-forms /100 ml	Fecal Strepto-cocci /100 ml	P. aeru-ginosa /100 ml	Fungi /100 ml	Salmonella			Parasites	Salmonella Collecting Pads
	Dur-ation	Amount Rainfall								100 ml	50 ml	10 ml		
June 19	0630-0930	6.3 mm	Manual	0935	2.8×10^5	4.7×10^3	4.0×10^4	1.0×10^3		Neg.	Neg.	Neg.	0 per 50 ml	Neg.
			Automatic		1.7×10^5	4.3×10^3	4.0×10^4	1.8×10^4		Neg.	Neg.	Neg.	0 per 50 ml	
			Manual	0955	1.2×10^5	4.5×10^3	6.0×10^4	1.6×10^3						
			Automatic		1.4×10^5	3.6×10^3	5.0×10^4	2.8×10^3						
			Manual	1015	2.0×10^4	7.5×10^3	2.8×10^4	1.5×10^3						
			Automatic		1.9×10^4	7.8×10^3	2.4×10^4	3.8×10^3						
July 11	1250-1900	5.6 mm			The sampler failed.									S. senftenberg
July 14	2430-0100	6.3 mm			The sampler failed.									S. newport
Aug. 11	0315-0600	3.81 mm	Automatic	0330	4.2×10^4	3.5×10^3	4.6×10^4	2.6×10^3	1.3×10^6	Neg.	Neg.	Neg.	1 Nematode per 100 ml	Neg.
Aug. 21	1230-1345	7.87 mm	Manual	1245	1.5×10^4	5.0×10^2	2.6×10^4	2.6×10^3	5.1×10^6	Neg.	Neg.	Neg.	0 per 50 ml	Neg.
				1310	1.8×10^7	1.4×10^4	2.3×10^4	7.4×10^3	3.1×10^5					
				1325	1.1×10^6	5.1×10^4	4.7×10^3	2.8×10^3	4.3×10^5					
				1340	4.3×10^5	3.8×10^4	2.5×10^3	5.5×10^3	2.4×10^5					
				1400	1.8×10^5	3.0×10^4	1.4×10^3	4.6×10^3	9.9×10^4					

Neg. = no growth.

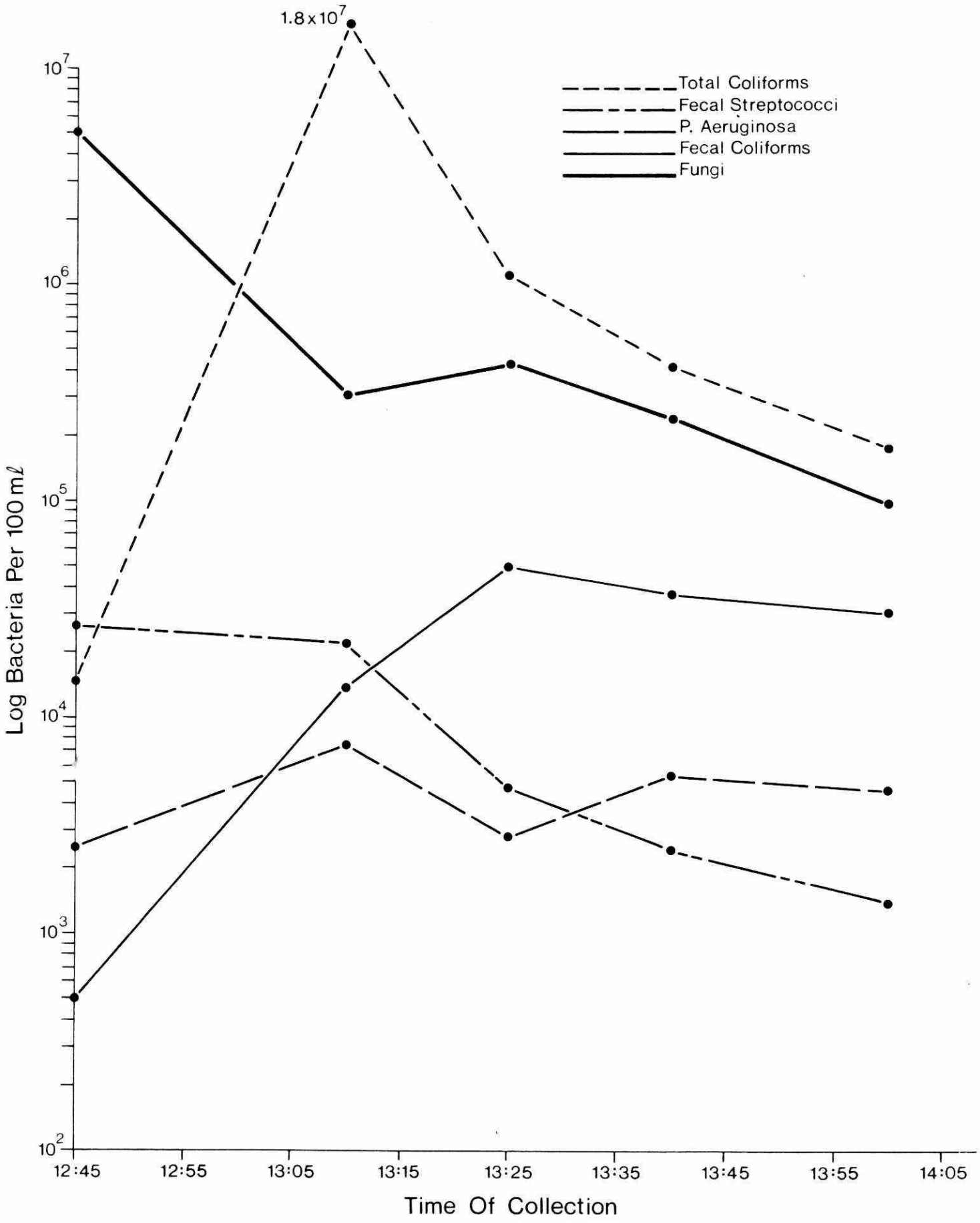


FIGURE 4. TYPICAL STORM RUNOFF, BACTERIOLOGICAL DATA, AUGUST 21, 1975, ALDERSHOT PLAZA

TABLE 3. INFILTRATION SAMPLES, MALVERN ROAD STORM SEWER, BURLINGTON

Date	Total Coliforms /100 ml	Fecal Coliforms /100 ml	Fecal Strepto-cocci /100 ml	P. aeru-ginosa /100 ml	Fungi /100 ml
June 16	$9.1x10^2$				
June 23	$4.1x10^3$	$2.1x10^1$	$2.9x10^2$	$3.0x10^1$	
July 2	$1.2x10^3$	$4.9x10^2$	$6.0x10^2$	$1.0x10^1$	
July 7	$7.4x10^3$	$3.1x10^2$	$1.7x10^3$	$5.8x10^2$	
July 16	$4.1x10^3$	$2.4x10^3$	$1.1x10^3$	$5.6x10^1$	
July 29	$2.0x10^3$	$2.7x10^3$	$2.5x10^3$	$3.6x10^1$	
Aug. 18	$1.9x10^2$	$1.3x10^3$	$1.6x10^2$	$2.4x10^2$	$1.0x10^3$
Sept. 9*	$2.3x10^3$	$8.7x10^2$	$3.1x10^2$	$9.0x10^1$	$1.1x10^3$

*Coliform Isolate Identification (33)
39% - E. coli
35% - Klebsiella
13% - Citrobacter
13% - Enterobacter

populations, when compared to Aldershot populations (Table 1), were 10^3 to 10^4 lower. Fecal coliform and fecal streptococcus populations were very similar to Aldershot, while Pseudomonas aeruginosa populations appeared to be 10^1 lower. Fungal populations, although only estimated twice were significantly lower at Malvern by 10^2 to 10^3.

Microbiological data presented in Figure 5 indicate a seasonal trend with maximum populations being reached in late June - early July and minimum populations occurring in mid-August.

Coliform isolate data indicate all isolates were entero-bacteriaceae with E. coli and Klebsiella genera predominating.

Similar to Aldershot, during one storm event a comparison was made between manually collected samples and those collected by the automatic sampler. The results of this study are shown in Figure 6 and Table 4. The presented data, with the exception of P. aeruginosa populations, indicate a very close correlation between hand-collected samples and machine-collected samples. It should be noted that these samples

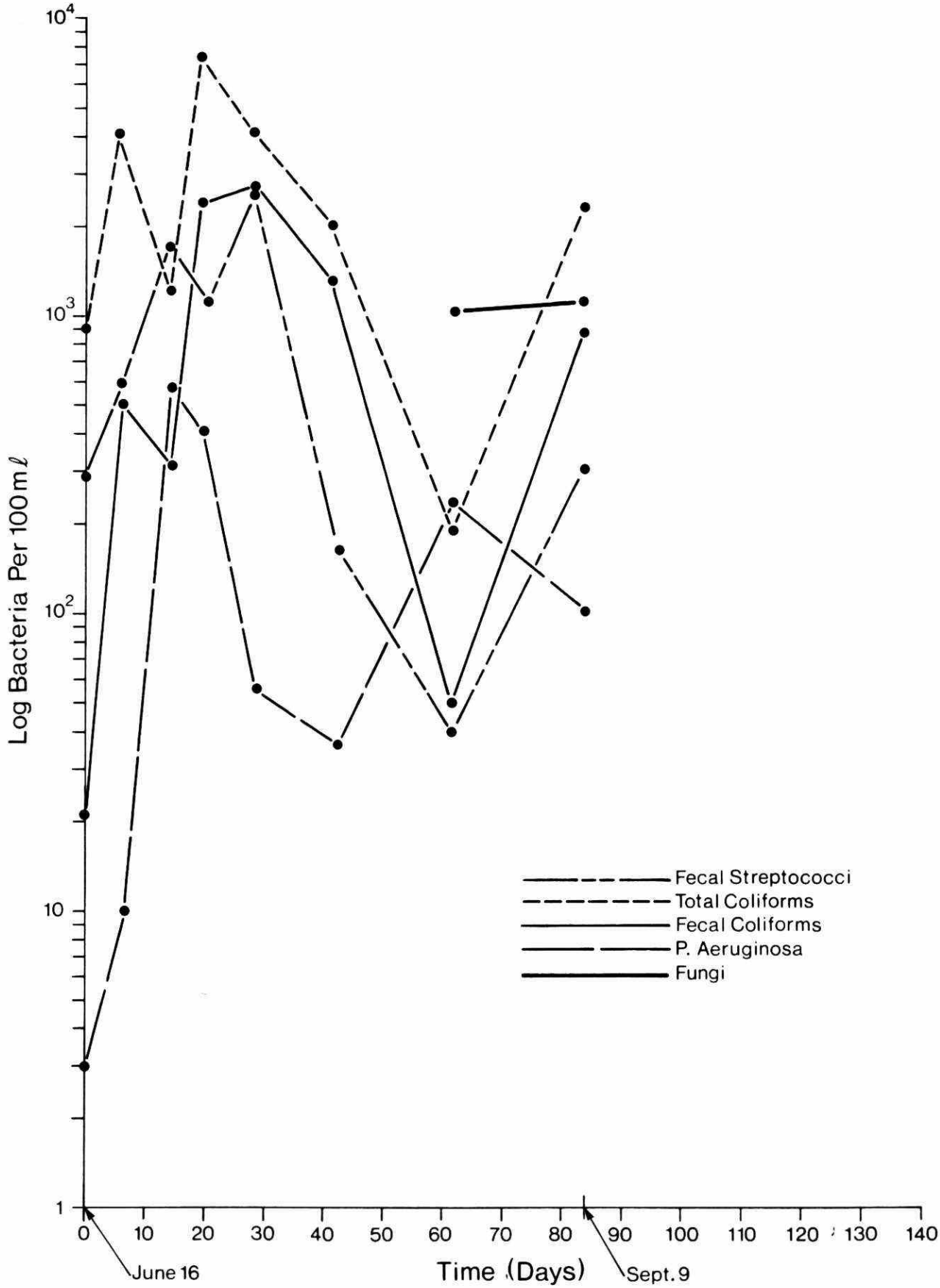
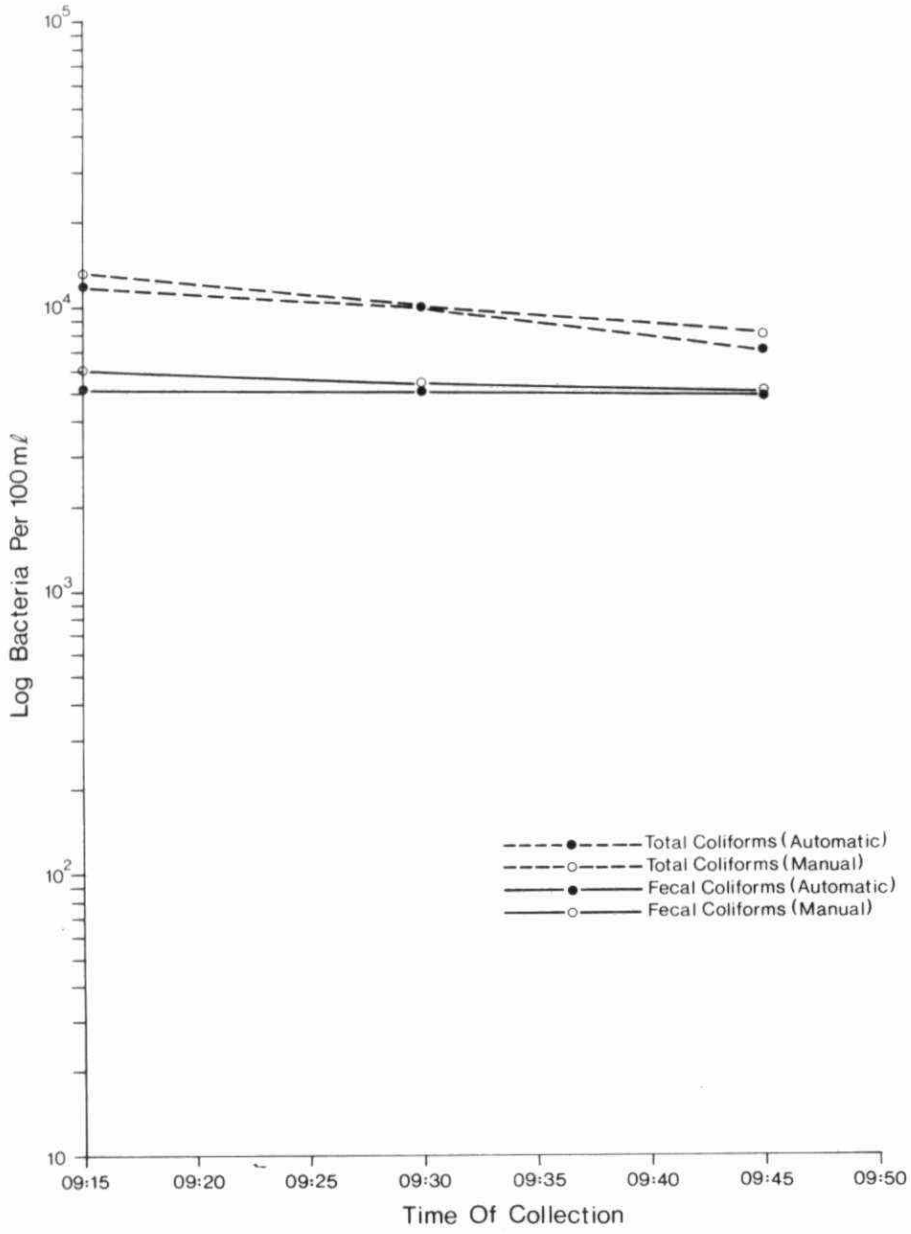


FIGURE 5. INFILTRATION DATA, MALVERN ROAD, BURLINGTON STORM SEWER, JUNE 16 - SEPTEMBER 9, 1975



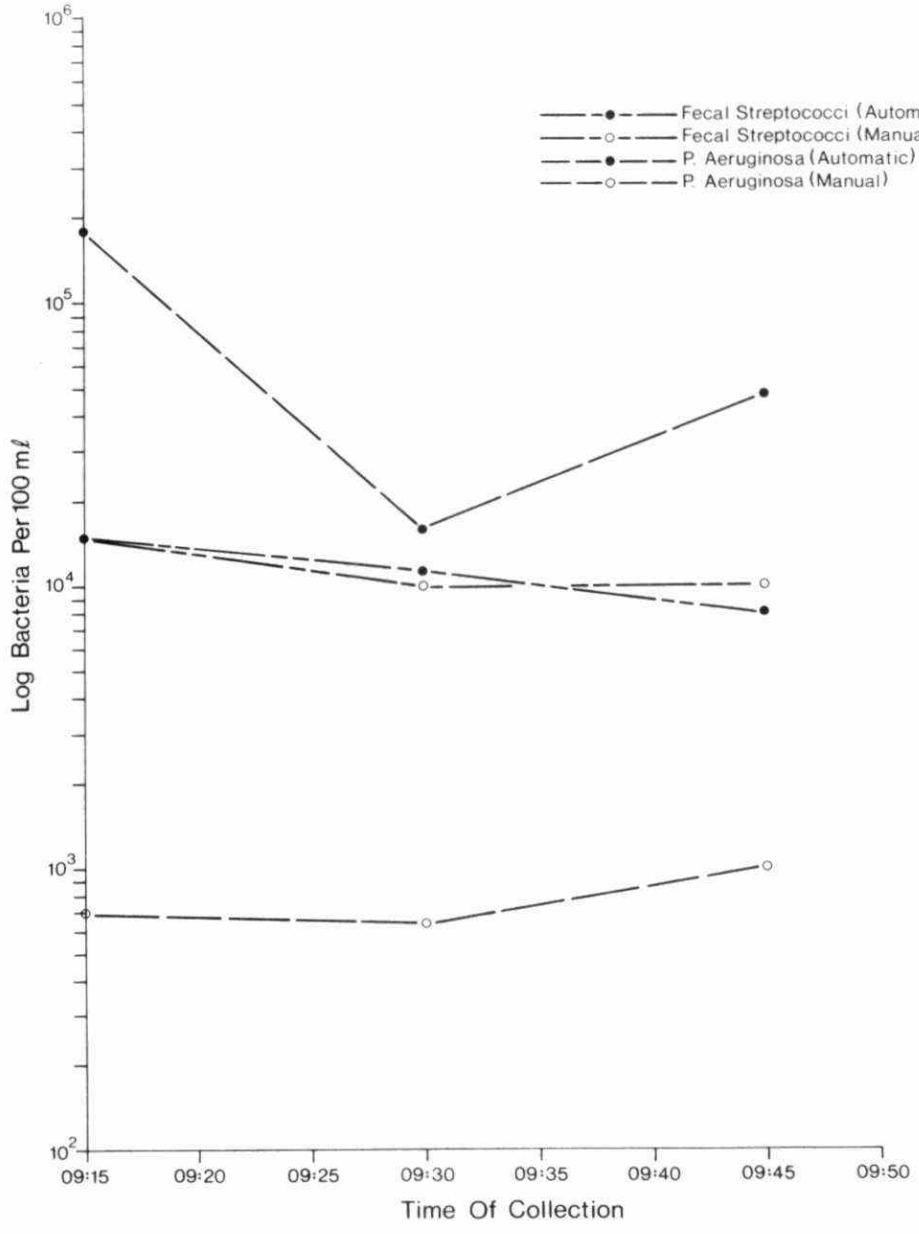


FIGURE 6. COMPARISON OF AUTOMATIC AND MANUAL SAMPLE COLLECTION PROCEDURES ON BACTERIAL POPULATIONS AT MALVERN ROAD, BURLINGTON

TABLE 4. MICROBIOLOGICAL DATA FROM MALVERN ROAD STORM SEWER, BURLINGTON, JUNE 19 - NOVEMBER 10, 1975

Date	Storm		Method of Sample Collection	Time of Sample Collection	Coli-forms /100 ml	Fecal Coli-forms /100 ml	Fecal Strepto-cocci /100 ml	P. aeru-ginosa /100 ml	Fungi /100 ml	Salmonella			Parasites	Salmonella Collecting Pads
	Dur-ation	Amount Rainfall								100 ml	50 ml	10 ml		
June 19	0630-0930	6.3 mm	Manual	0915	$1.3x10^4$	$6.0x10^3$	$1.5x10^4$	$7.0x10^2$		Neg.	Neg.	Neg.	0 per 50 ml	Neg.
			Automatic		$1.2x10^4$	$5.0x10^3$	$1.5x10^4$	$1.8x10^5$		Neg.	Neg.	Neg.		
			Manual	0930	$1.0x10^4$	$5.3x10^3$	$1.0x10^4$	$6.3x10^2$						
			Automatic		$1.0x10^4$	$5.0x10^3$	$1.1x10^4$	$1.6x10^4$						
			Manual	0945	$8.0x10^3$	$4.9x10^3$	$1.0x10^4$	$1.0x10^3$						
			Automatic		$7.0x10^3$	$4.9x10^3$	$8.0x10^3$	$4.7x10^4$						
June 24	15 min.	1.52 mm	Manual	Composite	$3.6x10^6$	$3.3x10^5$	$5.2x10^3$	$8.1x10^6$		Neg.	Neg.	Neg.	0 per 50 ml	S. Senftenberg
July 10	1315	5.84 mm	Manual	1315	$2.8x10^3$	$8.0x10^2$	$1.4x10^3$	$2.0x10^3$		Neg.	Neg.	Neg.	(2) Nematode per 100 ml	S. senftenberg
				1320	$3.3x10^5$	$9.8x10^3$	$3.4x10^4$	$6.9x10^4$						
				1330	$7.3x10^4$	$1.9x10^4$	$7.1x10^4$	$8.2x10^3$						
				1405	$2.1x10^4$	$1.6x10^3$	$1.5x10^4$	$2.1x10^3$						
July 14	2445	3.04 mm	Automatic	2455	$8.4x10^3$	$7.7x10^3$	$1.1x10^4$	$3.8x10^5$		Neg.	Neg.	Neg.	0 per 100 ml	Neg.
				0110	$1.4x10^3$	$1.0x10^2$	$6.2x10^3$	$3.7x10^5$						
				0130	$2.4x10^3$	$1.0x10^2$	$1.1x10^3$	$3.7x10^5$						
July 24	0550-0645	1.52 mm	Automatic	Beginning	$1.7x10^4$	$7.7x10^3$	$1.2x10^4$	$2.2x10^5$		Neg.	Neg.	Neg.	0 per 100 ml	Neg.
				End of Storm	$5.2x10^3$	$1.6x10^3$	$3.0x10^3$	$4.5x10^3$						
July 24	2100-2130	6.35 mm	Automatic	Composite	$2.6x10^4$	$1.2x10^4$	$2.9x10^3$	$4.7x10^5$		Neg.	Neg.	Neg.	0 per 100 ml	Neg.
July 27	1715-1815	5.84 mm	Automatic	1730	$7.9x10^4$	$1.4x10^4$	$3.6x10^3$	$1.6x10^6$		Neg.	Neg.	Neg.	0 per 80 ml	Neg.
				1800	$5.5x10^3$	$3.0x10^3$	$4.9x10^3$	$7.3x10^3$						
Aug. 6	0300-0730	4.06 mm	Automatic	0300	$7.8x10^4$	$1.2x10^4$	$3.7x10^3$	$5.5x10^5$	$6.4x10^4$	Neg.	Neg.	Neg.	0 per 100 ml	Neg.
				0430	$5.7x10^4$	$5.1x10^3$	$4.0x10^3$	$1.2x10^5$	$1.3x10^5$					
				0730	$2.3x10^4$	$1.0x10^3$	$3.7x10^3$	$9.3x10^4$	$1.2x10^5$					
Aug. 11	0345-0400	1.78 mm	Automatic	0345	$2.2x10^4$	$3.0x10^3$	$8.9x10^3$	$2.0x10^5$	$2.7x10^5$	Neg.	Neg.	Neg.	0 per 70 ml	Neg.
				0400	$1.6x10^4$	$1.2x10^3$	$8.8x10^3$	$1.4x10^5$	$7.5x10^4$					
Aug. 13	0945-1045	6.86 mm	Automatic	Composite	$2.6x10^4$	$1.3x10^4$	$2.0x10^4$	$1.1x10^7$	$1.0x10^7$	Neg.	Neg.	Neg.	0 per 60 ml	Neg.
Aug. 13	2115-2145	4.32 mm	Automatic	2115	$3.4x10^3$	$3.0x10^2$	$2.0x10^2$	$1.6x10^6$	$8.6x10^5$	Neg.	Neg.	Neg.	(2) Nematode per 45 ml	Neg.
				2130	$1.2x10^5$	$4.6x10^3$	$8.9x10^3$	$8.9x10^5$	$4.0x10^6$					
				2145	$1.8x10^3$	$1.2x10^3$	$6.3x10^3$	$4.3x10^5$	$4.6x10^6$					
Aug. 21	1230-1400	6.35 mm	Automatic	1230	$8.4x10^4$	$1.3x10^4$	$4.3x10^4$	$9.7x10^5$	$7.8x10^5$	Neg.	Neg.	Neg.	0 per 95 ml	Neg.
				1315	$5.6x10^6$	$1.5x10^4$	$1.0x10^4$	$3.0x10^5$	$3.4x10^5$					
				1400	$4.1x10^4$	$2.6x10^3$	$9.0x10^3$	$8.7x10^4$	$2.4x10^4$					
Oct. 1	0930-1120	2.54 mm	Automatic	Composite	$3.4x10^4$	$8.1x10^3$	$4.8x10^3$	$3.1x10^4$	$1.2x10^5$	Neg.	Neg.	Neg.	0 per 30 ml	Neg.
Oct. 19	2130-0730	? +2.5 mm	Automatic	2130	$7.8x10^4$	$2.2x10^4$	$3.0x10^3$	$7.0x10^2$	$1.2x10^6$	Neg.	Neg.	Neg.	0 per 100 ml	Neg.
				0230	$1.2x10^4$	$4.2x10^4$	$4.2x10^4$	$4.0x10^2$	$5.0x10^2$	$2.3x10^5$				
				0430	$1.7x10^4$	$5.5x10^3$	$8.0x10^2$	$7.7x10^3$	$5.2x10^5$					
Nov. 10	0330-1145	9.14 mm	Automatic	0330	$1.1x10^4$	$6.1x10^3$	$1.7x10^4$	$3.0x10^2$	$3.0x10^5$	Neg.	Neg.	Neg.	0 per 100 ml	Neg.
				0600	$5.7x10^3$	$3.6x10^3$	$1.5x10^4$	$1.0x10^2$	$3.5x10^5$					
				0830	$4.4x10^3$	$4.0x10^3$	$1.5x10^4$	$8.0x10^2$	$1.2x10^5$					
				1100	$3.2x10^3$	$2.7x10^3$	$1.5x10^4$	$1.0x10^2$	$1.7x10^5$					

Neg. = no growth.

were collected from the end of the storm, and the automatic sampler was triggered by hand.

The majority of samples analyzed from Malvern were collected by automatic sampler (Table 4). Many of the storms occurring during the sample collection period were short and intense, and in many instances the first samples were collected only minutes after the storm occurred. At other times several minor cloudbursts occurred, triggering the automatic sampler at various intervals, e.g., August 6, and making maximum population occurrence estimations unreliable. Maximum populations generally occurred within the first 15 minutes of the storm's initiation.

Fungal populations were surprisingly high, varying from 10^5 to 10^7 per 100 ml. Since the procedures used do not differentiate between pathogenic and non-pathogenic fungi, it is possible that these elevated densities indicate a previously unsuspected potential health hazard. Similarly, P. aeruginosa populations, which frequently reached 10^5 and 10^6 levels, and over 10^7, and are known health hazards, indicate an unsuspected health hazard in the storm runoff waters.

No obvious seasonal trend can be seen in Malvern data, with the possible exception of P. aeruginosa populations, which appear to reach minimum levels in late October and November.

In Figure 7 the data from an ideal, typical storm is displayed and it can be seen that coliforms and P. aeruginosa populations reach their maximum densities within the first five minutes of a storm event, and within the first 15 minutes, fecal coliform and fecal streptococcus populations reach their maximum levels.

During two events, June 24 and July 10, S. senftenberg was isolated by means of the collecting pad procedure. The June 24 event was a very short, heavy rain whose storm waters contained some of the highest concentrations of coliforms, fecal coliforms and P. aeruginosa found during the Malvern study.

During two events, July 10 and August 13, nematodes probably belonging to the order Rhabditida were observed in the storm waters.

Microbiological data obtained from sediment samples collected during storm events and an infiltration sample collection period are presented in Table 5. The data indicate that sediments are a good

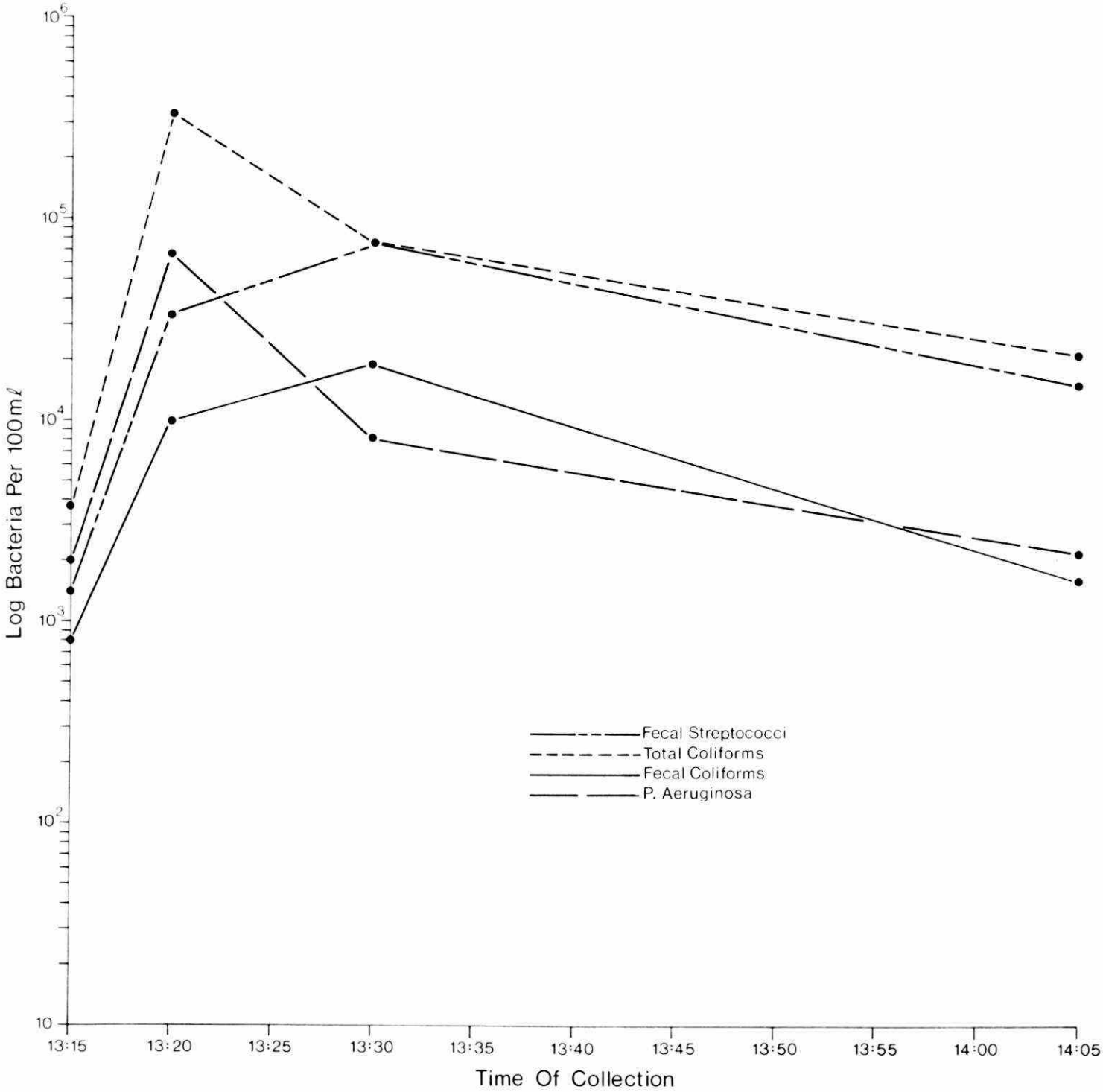


FIGURE 7. STORM RUNOFF BACTERIOLOGICAL DATA, JULY 10, 1975, MALVERN ROAD, BURLINGTON

TABLE 5. SUMMARY OF SEDIMENT SAMPLE DATA

Sampling Site	Date	Total Coliforms /10 gm Wet wt.	Fecal Coliforms /10 gm Wet wt.	Fecal Strepto-cocci/10 gm Wet wt.	P. aeru-ginosa /10 gm Wet wt.	Fungi /10 gm Wet wt.	Salmonella /10 gm Wet wt.
Malvern Road, Burlington	Aug. 11	$6.3x10^4$	$7.3x10^3$	$9.1x10^3$		$8.4x10^4$	Negative
	Sept. 9	$4.7x10^4$	$3.0x10^2$	$1.0x10^2$		$9.0x10^3$	Negative
	Sept. 22	$1.8x10^3$	$1.1x10^2$	$4.9x10^2$	$1.8x10^3$	$4.9x10^4$	Negative
	Oct. 1	$4.0x10^3$	$1.5x10^3$	$8.4x10^2$	$3.0x10^2$	$9.5x10^4$	Negative
Brucewood, Toronto	Sept. 18	$1.0x10^3$	$2.0x10^2$	$4.0x10^2$	$1.4x10^2$	$7.0x10^3$	Negative

reservoir for the various microbial populations. No salmonellae were found in these sediments.

5.3 Brucewood

All samples collected at the Brucewood site were collected manually. Since this site was selected during mid-program, only one infiltration sample was collected. Data from this single sample are presented in Table 6. Microbial populations in this sample were very much lower than those obtained from Aldershot and Malvern samples. Coliform isolate data revealed a high level of Citrobacter species, which are typical of rural countrysides [9].

TABLE 6. INFILTRATION SAMPLE, BRUCEWOOD STORM SEWER, TORONTO SEPTEMBER 18, 1975

Parameter	Microbial Densities per 100 ml
Total coliforms	$1.5x10^2$
Fecal coliforms	$2.7x10^1$
Fecal streptococci	$5.2x10^1$
P. aeruginosa	$2.0x10^1$
Fungi	$1.3x10^2$

Coliform Isolate Identification (34)	6%	- Aeromonas
	41%	- Citrobacter
	26%	- E. coli
	18%	- Klebsiella
	9%	- Enterobacter

Storm runoff data presented in Table 7 and in Figure 8 (a-d) indicate microbial populations remained fairly steady throughout the sample collection period. Only P. aeruginosa populations tended to fluctuate during storms, one time peaking at the end of the sample collection period (October 1), and during another event, reaching minimum populations at the end of the storm. However, careful examination of the time at which the first sample was collected reveals that at least 24 minutes passed before the first sample was collected. Thus, according to Malvern data, peak microbial concentrations had passed and only the tail-end of the storm had been observed. Even though peak microbial levels were not observed at Brucewood, the storm data obtained showed a greater difference in microbial densities between storm water and infiltration water than occurred at the Aldershot and Malvern sites.

In Figure 9, geometric means of all storms by time of collection (with zero hour being time of first sample collection) are presented. Again, from this presentation it can be seen that only P. aeruginosa populations showed any significant variations over the sample collection period.

Coliform isolate data presented in Table 8 show an increase in E. coli levels in storm water and the maintenance of Citrobacter levels. Thus storm water coliform populations tend to support infiltration data, i.e., rural land runoff augmented by fecal organisms (E. coli).

Attempts to isolate salmonella and parasites (Table 9) at Brucewood were all negative. These negative results may be an indication of missed initial flows and peak microbial populations.

An infiltration sediment sample revealed microbial densities on the same order of magnitude as those obtained from Malvern samples (Table 5).

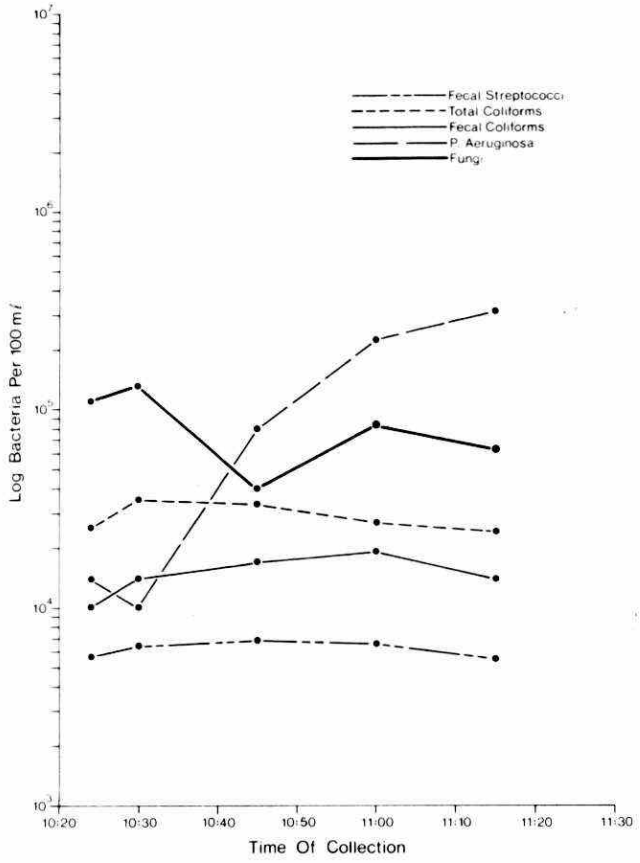
5.4 Fecal Sterols

Coprostanol levels from all sites, infiltration and storm waters, were below detection limits (0.05 micrograms per one litre) (Table 9). Thus, in the samples collected, there was no detectable fecal material.

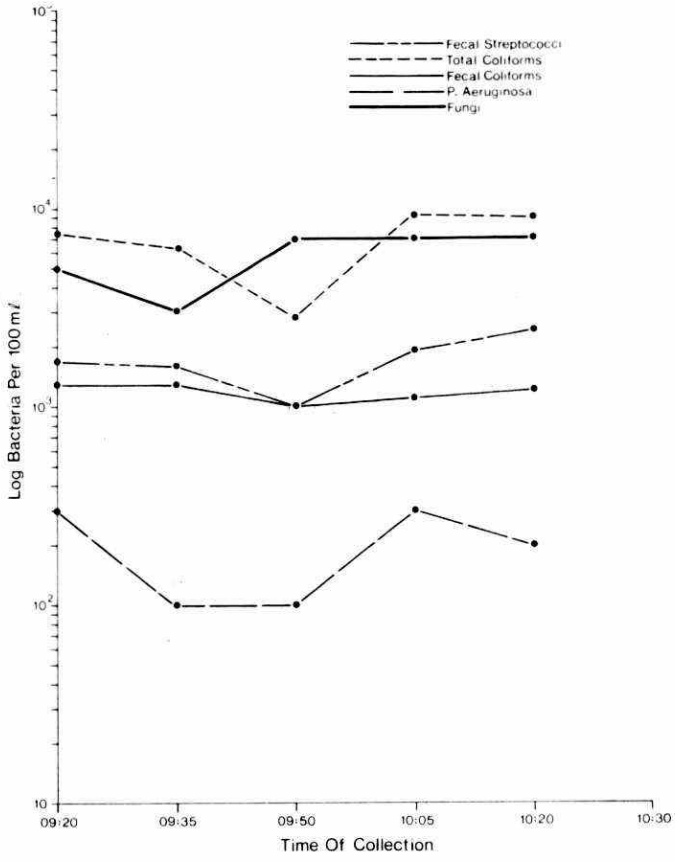
TABLE 7. MICROBIOLOGICAL DATA FROM BRUCEWOOD STORM SEWER, TORONTO, OCTOBER 1 - NOVEMBER 27, 1975

Date	Storm		Method of Sample Collection	Time of Sample Collection	Coli-forms /100 ml	Fecal Coli-forms /100 ml	Fecal Strepto-cocci /100 ml	P. aeru-ginosa /100 ml	Fungi /100 ml	Salmonella			Parasites	Salmonella Collecting Pads
	Dur-ation	Amount Rainfall								100 ml	50 ml	10 ml		
Oct. 1	1000-1325	4.06 mm	Manual	1024	2.6×10^4	1.0×10^4	5.7×10^3	1.4×10^4	1.1×10^5	Neg.	Neg.	Neg.	0 per 100 ml	Neg.
				1030	3.5×10^4	1.4×10^4	6.5×10^3	1.0×10^4	1.3×10^5					
				1045	3.3×10^4	1.7×10^4	6.9×10^3	8.0×10^4	4.0×10^4					
				1100	2.7×10^4	1.9×10^4	6.5×10^3	2.2×10^5	8.3×10^4					
				1115	2.4×10^4	1.4×10^4	5.5×10^3	3.1×10^5	6.2×10^4					
Oct. 20	0845-1030	.76 mm	Manual	0920	7.6×10^3	1.3×10^3	1.7×10^3	3.0×10^2	5.0×10^3	Neg.	Neg.	Neg.	0 per 100 ml	Neg.
				0935	6.3×10^3	1.3×10^3	1.6×10^3	1.0×10^2	3.0×10^3					
				0950	2.8×10^3	1.0×10^3	1.0×10^3	1.0×10^2	7.0×10^3					
				1005	9.1×10^3	1.1×10^3	1.9×10^3	3.0×10^2	7.0×10^3					
				1020	8.8×10^3	1.2×10^3	2.4×10^3	2.0×10^2	7.0×10^3					
Nov. 10	0355-1130	20.0 mm	Manual	0945	1.7×10^4	7.3×10^3	9.7×10^3	1.3×10^2		Neg.	Neg.	Neg.	0 per 100 ml	Neg.
				0955	2.2×10^4	5.1×10^3	1.0×10^4	1.0×10^2						
				1005	1.4×10^4	7.1×10^3	1.1×10^4	7.0×10^2						
				1015	1.5×10^4	3.5×10^3	1.1×10^4	8.0×10^2						
				1025	1.4×10^4	6.0×10^3	1.1×10^4	1.2×10^2						
				1035	9.0×10^3	4.5×10^3	1.2×10^4	3.0×10^1						
				1045	8.0×10^3	5.1×10^3	1.3×10^4	6.0×10^1						
Nov. 27	0151-1001	16.0 mm	Manual	0930	5.8×10^3	1.7×10^3	4.3×10^3			Neg.	Neg.	Neg.	0 per 100 ml	Neg.
				0945	8.5×10^3	3.8×10^3	5.9×10^3							
				1000	8.1×10^3	3.0×10^3	2.3×10^3							
				1015	9.9×10^3	3.3×10^3	1.9×10^3							
				1030	7.3×10^3	4.1×10^3	2.0×10^3							

Neg. = no growth.



STORM RUNOFF, BACTERIOLOGICAL DATA
OCT. 1, 1975 BRUCEWOOD, TORONTO



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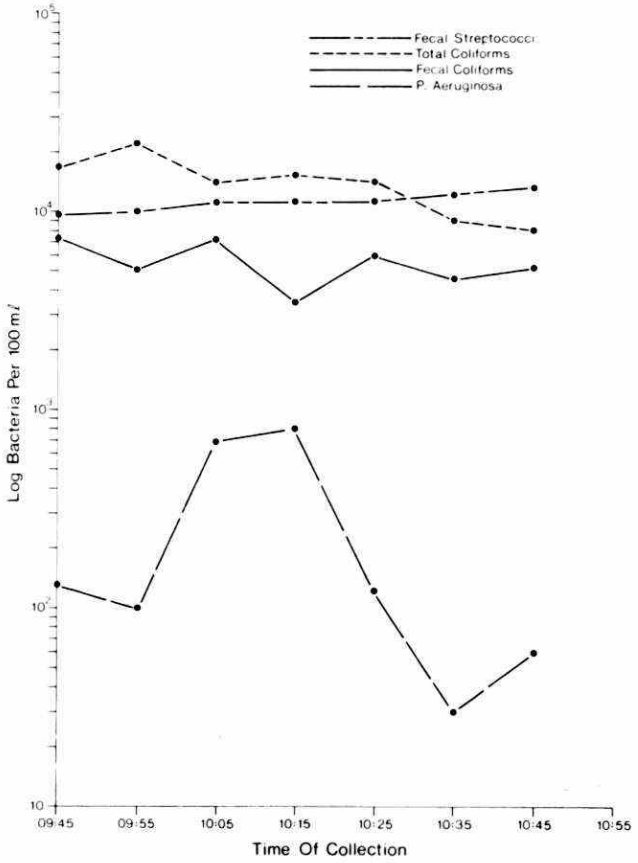
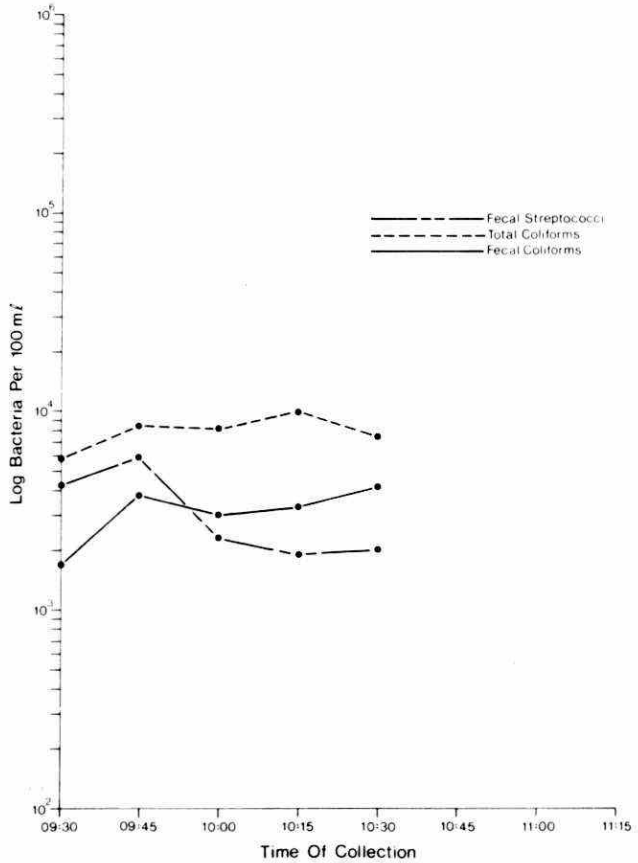
OCT. 20, 1975 BRUCEWOOD, TORONTO



STORM RUNOFF, BACTERIOLOGICAL DATA
NOV. 10, 1975, BRUCEWOOD, TORONTO.



STORM RUNOFF, BACTERIOLOGICAL DATA
NOV. 27, 1975 BRUCEWOOD, TORONTO

FIGURE 8. STORM RUNOFF, BACTERIOLOGICAL DATA, BRUCEWOOD, TORONTO

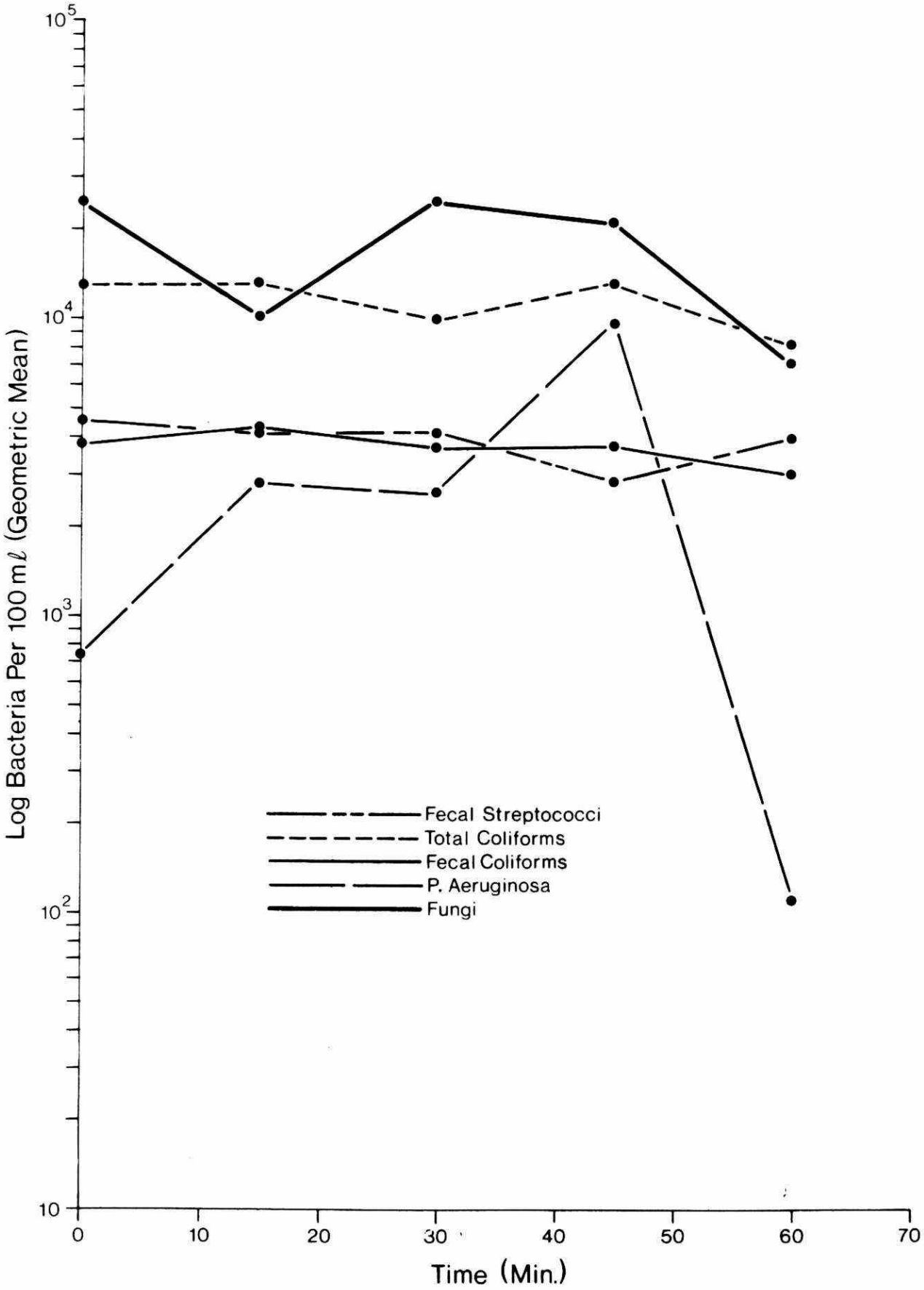


FIGURE 9. VARIATIONS IN MICROBIOLOGICAL POPULATIONS (Expressed As Geometric Means) DURING SPECIFIC SAMPLING TIMES FROM FOUR STORM EVENTS (BRUCEWOOD)

TABLE 8. COMPARISON, COLIFORM BIOTYPE DISTRIBUTION IN BRUCEWOOD INFILTRATION AND STORM RUNOFF SAMPLES

Genera	Type of Sample	
	Infiltration	Storm runoff
Aeromonas	6%	0%
Citrobacter	41%	33%
Enterobacter	9%	12%
Klebsiella	18%	6%
E. coli	26%	49%

TABLE 9. FECAL STEROL CONCENTRATIONS, ALL SAMPLES

Date	Source	Sample	Coprostanol (micrograms per litre)
June 23	Aldershot Plaza	Infiltration	<0.05
July 7	Aldershot Plaza	Infiltration	<0.05
Aug. 21	Aldershot Plaza	Storm Runoff	<0.05
June 23	Malvern Road	Infiltration	<0.05
July 7	Malvern Road	Infiltration	<0.05
July 10	Malvern Road	Storm Runoff	<0.05
July 14	Malvern Road	Storm Runoff	<0.05
July 24	Malvern Road	Storm Runoff	<0.05
July 25	Malvern Road	Storm Runoff	<0.05
Nov. 27	Brucewood	Storm Runoff	<0.05

6. DISCUSSION

The major problem encountered during this study was the collection of samples suitable for microbiological analysis and for typifying the various stages of storm water runoffs. During the study, all automatic sampling devices failed at one time or other. When they were operational, initial or zero hour samples often were not collected because water levels did not rise sufficiently high, quickly enough to trigger the sampler mechanism. At other times, during an all-day or all-night rain, several erratic, short, heavy downpours occurred, thus triggering haphazard sample collection.

Thus, with the automatic sampling equipment it was very difficult to follow some events and to establish a zero hour. A proper set of data could be collected only from those events which started with a heavy downpour and maintained at least a 4 cm water level rise in the storm mains.

Once the automatic samples were collected, another major problem occurred. This was the lack of proper sample preservation. If the event occurred outside of normal working hours, the samples sat until 8 or 9 a.m. This holding period varied from 0 to 18 hours. During this time, microbiological populations and chemical parameters were constantly changing. Thus, the reliability of the presented data must be suspect.

Manual sample collection would appear to be the answer. However, even here, problems were encountered. One of the main problems, again, was the collection of zero hour samples. It was extremely difficult to have a sample collector at the weir when the storm's effects were first felt because the collector often had to drive a considerable distance to reach the sampling site. This was an especially serious problem when the storm started with a sudden heavy downpour. During localized storms, the sampler would not even be aware of an event taking place.

Another problem which occurred during manual sample collection was that samples were usually only collected during the 8 a.m. to 5 p.m. working day. Nevertheless, with all its inherent problems, manual sample collection may be the only satisfactory way of collecting accurate storm runoff data, unless automatic samplers could be adjusted to collect

samples from any flow greater than infiltration flows, in sterile refrigerated containers.

Infiltration data indicate that Aldershot and Malvern samples contained much higher microbial populations than Brucewood samples. Furthermore, Aldershot microbial populations, especially coliforms, reached dilute sewage levels, probably as a direct result of the completely impervious nature of the catchment area. Since it has always been assumed that pathogens, i.e., salmonella, shigella, viruses, etc., co-exist with high concentrations of fecal coliforms [11], it can be seen that even the infiltration samples contain potential health hazards. However, the source of these fecal coliforms and suspected pathogens is difficult to ascertain in the infiltration samples.

Because of sample collection problems and storm vagaries it was extremely difficult to establish a specific time when maximum microbial populations occurred in storm water runoffs during an average storm. From the collected data it would appear that most microbial populations reached their maximum levels within the first 45 minutes of the beginning of an event.

Traditional indicator densities, such as coliforms, fecal coliforms and fecal streptococci, approached dilute sewage concentrations during each event, thus re-emphasizing the health hazards of pathogenic bacteria and viruses [1] contained within these storm runoff waters. It must also be assumed that human and animal pathogens also increased in density and frequency to an unknown degree during each storm event.

One of the indicator organisms used in this study, Pseudomonas aeruginosa, is also a pathogen. This organism has been implicated in eye, ear and upper respiratory tract infections in bathers. Thus, the increased P. aeruginosa populations discharging into receiving waters which are frequently used for recreation constitute an elevated health risk not previously suspected [2,3,4].

The mycological procedures used during this study did not differentiate between invasive, pathogenic fungi and saprophytic fungi. It is known that both types will grow on the media used. The striking increase seen in fungal populations during the storm events (Tables 2 and 7) should be cause for concern, especially as many of the fungi invade

through skin contact. Storm runoff waters are therefore contributing another previously unsuspected group of potential pathogens to receiving and recreational waters.

Isolate collection data indicate the uniqueness of each sampling site. The Aldershot coliform isolates were found to contain 50% pseudo-coliforms and only 5% E. coli, signifying very low fresh fecal contamination. Malvern isolates were a more general mix, containing a moderate E. coli population, typical of fresh fecal material, probably from the resident pet and wild animal population, and a small number of Citrobacter sp. typically associated with soil. The Brucewood area was typified by high Citrobacter sp. levels which are strongly supported by the wooded semi-rural setting of this catchment area. It would be interesting to initiate a more detailed isolate study in the various catchment areas to ascertain if the endemic enterobacteriaceae infiltration populations consistently typify the catchment area.

The finding of salmonellae in the storm runoff waters by the collection pad technique confirms the presence of health hazard organisms in these waters. The inability to find salmonellae in 100-ml aliquots of storm water indicates: (a) that salmonella concentrations are less than one organism per 100 ml; (b) that salmonella populations are not homogeneous throughout storm runoff waters and, since they are directly related to fecal material, are only found in slugs of water; and (c) that samples for salmonella isolation and enumeration must be collected more frequently and in larger quantities to enumerate these heterogeneously dispersed pathogens.

The inability to find any typical human parasites in the storm runoff waters does not necessarily indicate their absence. Since the fecal material contaminating the storm runoff waters was either animal or bird related, it is suspected that animal parasites may be present. However, the heterogeneity of the storm water and relative infrequency of these organisms prevented sufficiently frequent sample collections of large enough size to isolate these sporadically appearing organisms.

The lack of detectable coprostanol levels from infiltration and storm water samples at all sites indicates the very low concentration of fresh feces in these waters. When present, they are probably found in slugs. The impact of fecal slugs is difficult to assess through chance

sampling. This hypothesis is supported by the salmonella data, where pathogens were isolated only by continuous sampling, i.e., collecting pads.

The nature of the pathogen and fecal sterol distribution patterns and infiltration microbiological background levels indicates that the majority of the microbial populations were from land once polluted with feces, where the bacteria have become endemic under proper nutrient and growing conditions. The incidence of true pathogens, salmonella and P. aeruginosa, and the suspected but not enumerated viruses and pathogenic fungi, is probably the result of fresh fecal deposition. P. aeruginosa may be an exception because these organisms can readily multiply under natural conditions. To obtain a more realistic estimation of health hazards associated with storm runoff waters, it may be appropriate to sample more frequently and in larger aliquots for these pathogens, both in infiltration waters and storm waters.

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MICROBIOLOGICAL CHARACTERISTICS OF STORM WATER RUNOFFS AT EAST YORK (TORONTO) AND GUELPH SEPARATE STORM SEWERS

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ABSTRACT

A six-month study was conducted to determine the microbiological characteristics of storm water runoffs at East York (Toronto) and Guelph separate storm sewers. The results indicated that storm water discharges from these two systems contain high levels of pollution indicator bacteria and some pathogenic bacteria. The pollution appears to be predominantly of non-human origin and is mainly derived from animal wastes. In general, the runoffs resembled dilute raw wastewaters in microbiological composition and represent a public health risk.

The results of this study also showed the seriousness of urban storm water runoffs as a major factor in "nonpoint" source pollution of surface waters.

RÉSUMÉ

Une étude de six mois a servi à évaluer les propriétés microbiologiques des eaux de ruissellement des égouts séparatifs d'East York (Toronto) et de Guelph. Les résultats montrent que les eaux pluviales de ces deux réseaux renferment de fortes concentrations de bactéries indicatrices de pollution et certaines bactéries pathogènes. La pollution, qui semble être en majeure partie étrangère à la population humaine, provient principalement d'excréments animaux. En général, les eaux de ruissellement, dont la composition microbiologique ressemble à celle d'eaux résiduaires brutes diluées, menacent la santé du public.

Les résultats de l'étude montrent aussi que les eaux de ruissellement urbaines sont une importante source non ponctuelle de pollution des eaux de surface.

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CONCLUSIONS

1) Discharges from the Barrington Avenue (Toronto) and the Woodlawn Road (Guelph) separate storm sewer systems contained significant quantities of fecal pollution indicator bacteria. The total coliform densities ranged from 3,000 to 1,190,000 per 100 ml. Fecal coliform levels varied from 200 to 560,000 per 100 ml. Fecal streptococci densities, which were generally equal to or greater than fecal coliforms, ranged between 3,000 and 620,000 per 100 ml.

2) Remarkably high populations of fungi were found in storm water discharges at both sites. The concentrations fluctuated from 140,000 to 12,000,000 per 100 ml.

3) Opportunistic pathogenic bacteria viz. Pseudomonas aeruginosa and Staphylococcus aureus were infrequently isolated from runoffs at both storm sewers. The densities of these two organisms varied from 1 to 630 and 1 to 100 per 100 ml, respectively.

4) During this study, salmonellae were consistently detected in discharges from the Toronto and Guelph storm sewers. In several instances, these pathogens were readily isolated from as little as 10-ml aliquots of storm waters. The salmonella isolates belonged to four different serotypes, including S. haardt, S. saint-paul, S. tennessee and S. typhimurium.

5) The strikingly high concentrations of microbial populations found in sediments from these two systems indicate that such sediments constitute a reservoir for various microorganisms which ultimately find their way into storm water runoffs.

6) No typical patterns of the distribution of indicator bacteria or of pathogenic bacteria in storm water runoffs could be established during this study. The peak microbial populations occurred either at the initial stages (0 to 15 minutes), middle (20 to 40 minutes) or the tail-end (45 to 60 minutes) of the sampling period of different storms monitored. The unpredictable and intermittent release of microorganisms in storm waters suggests that any effect of "initial flushing" on

microbiological quality of the discharges was minimal during an individual storm.

7) The Barrington Avenue storm sewer showed higher levels of microbial populations and yielded greater numbers of pathogenic bacteria than the Woodlawn Road system. In addition, some seasonal differences in the bacterial densities of pollution indicator bacteria were noted at the Toronto site.

8) The data indicate that fecal pollution in these two separate storm sewers is predominately of non-human origin. Fecal material from animals and birds washed from the streets during rain storms appears to be the major source of pollution. In addition, runoffs from vegetation and soil may also contribute fluctuating levels of indicator bacteria to storm water discharges.

9) The levels of microbial populations in storm water runoff were strikingly high throughout the entire sampling period and many times approached densities found in raw sanitary wastewaters and therefore constitute a health hazard. The implied public health risk is substantiated by the recovery of pathogenic and potentially pathogenic bacteria in discharges from these two storm sewers. All four Salmonella serotypes found in this study have also been isolated from humans in Canada during recent years.

10) This study shows that the discharges from separate storm sewers can be a major source of pollution to receiving surface waters and reservoirs used for recreational purposes. Definite remedial measures must be applied to prevent public health risks and deterioration of surface water quality.

1 INTRODUCTION

During recent years there has been a growing recognition of the importance of storm water runoff as a major source of pollution in natural surface waters. Several studies have been conducted to determine the quantitative and qualitative characteristics of storm water discharges and their impact on receiving waters [1-16].

Weibel et al [2] reported that 90 percent of all storm water samples from a separately sewered urban area had coliform, fecal coliform and fecal streptococcus counts exceeding 2900, 500 and 4900 per 100 ml, respectively. In ten percent of the samples, the levels of these groups were greater than 460 000, 76 000 and 110 000, respectively.

Burm and Vaughan [5] found that the total coliform levels in the runoff from separate storm sewers were about one-tenth of those in combined sewers where median monthly values were as high as 37 000 000 per 100 ml. Fecal coliform densities in separate storm sewer runoffs were rarely more than 20 percent of the total coliform population. Also, the combined sewer discharges contained 40 times as many fecal coliforms as were detected in separate storm sewers. They found that in both types of sewer systems fecal streptococcus densities were similar and were generally equal to or greater than the fecal coliform counts. Similarly, Benzie and Courchaine [6] found that the discharges from a combined sewer contained considerably larger quantities of total coliforms, fecal coliforms and fecal streptococci than those from a separate storm sewer system.

Burm [7] showed that total coliform, fecal coliform and fecal streptococcus densities in a large amount of the receiving water increased considerably as a result of overflows from combined sewers after a moderate rainstorm. In receiving waters located above the overflows, however, the effects of the storm were negligible. He indicated that the relative increases in bacterial densities were dependent on the distance between the outfall and the sampling points. With the increasing distance from the discharge point, the increases in bacterial levels became less pronounced.

Geldreich et al [10] reported similarities in bacteriological composition between storm water runoff from cultivated farms and runoffs

from city streets, a suburban business district storm drain, and a wooded hillside. Strator et al [12] showed that runoff from street surfaces was heavily contaminated and in many respects resembled sanitary wastewater. Using calculations based on a hypothetical city, they indicated that the runoff from the first hour of a moderate to heavy storm (1.27 cm/hr) would contribute a substantially greater pollution load than would the same city's sanitary waste during the same event.

In addition to fecal pollution indicator bacteria, the presence of pathogenic bacteria has also been reported in storm water. Evans et al [9] demonstrated the existence of a potential health hazard by isolating Salmonella thompson (at a level of 4 500/100 ml) in a storm water sample from an urban business district separate storm sewer. This storm water sample contained 3 800 000 total coliforms, 450 000 fecal coliforms, and 370 000 fecal streptococci per 100 ml.

The fecal pollution bacteria in storm water runoff from the separate storm sewers have been shown to be primarily of non-human origin, while those in combined sewers are of human origin [2,5,6,9,10]. The non-human fecal contamination in separate storm water systems is mainly derived from rodents, cats, dogs and birds [10].

Since rainwater contains insignificant levels of bacteria, its major contamination occurs on contact with the land environment, creating a potential pollution problem in the resulting runoff [10]. Among the many and different sources of pollution in urban storm water runoff are: street debris and litter, soil-chemical mixtures washed from surrounding open lands, garden fertilizers, herbicides and soil conditioners, air pollutants, deicing chemicals, dirt, oil, grease and other contaminants washed from automobiles, combustion byproducts and animal waste [10,12,17,18,19,20,21,22,23].

Relatively few reported data are available on the microbiological quality of storm water runoff in Canada. As a part of the Canada-Ontario Agreement (COA), a study was designed to provide such information by examining several storm sewers in central Ontario. The main objectives of this investigation were: (i) to determine the incidence and distribution of pollution indicator microorganisms and selected pathogens in

runoff from storms of different intensities and duration at two separate storm sewers, (ii) to determine the probable sources of contamination, and (iii) to evaluate the significance of storm water pollution and associated potential health hazards.

2. STUDY AREAS

Descriptions of the two storm water runoff-producing drainage areas examined in this study are given below.

2.1 Barrington Avenue (East York), Toronto

The Barrington site is located in the Borough of East York and includes part of the City of Toronto (Figure 1). With the exception of five or six corner stores, the area is entirely composed of single-family residential housing ranging from 50 to 70 years in age. The site is 100% separate sewers with a total drainage area of 22.7 hectares (56.0 acres). The area containing buildings not connected to the storm sewer system is approximately 5.3 hectares (13.0 acres). Private lawns, walkways, driveways, roads and public sidewalks constitute the net area (17.4 hectares, 43.0 acres) contributing to storm water runoff. The Chisholm Avenue and Main Street storm sewers flow to the trunk sewer on Lumsden Avenue, which in turn carries the flow to a 10-metre dropshaft at the end of Barrington Avenue and eventually northward to Massey Creek through a 152-cm circular pipe (Figure 1).

2.2 Woodlawn Road, Guelph

The Woodlawn Road site is approximately 70.8 hectares (177.0 acres) and is located near the Speed River at the northwest suburban area of the City of Guelph at 80^{o} - 16' longitude and 43^{o} - 34' latitude. It comprises almost entirely new single and multiple family housing. Some commercial sites are also present in the drainage area. The area has 100% separate storm sewers.

SCALE : 1 centimetre = 60 metres

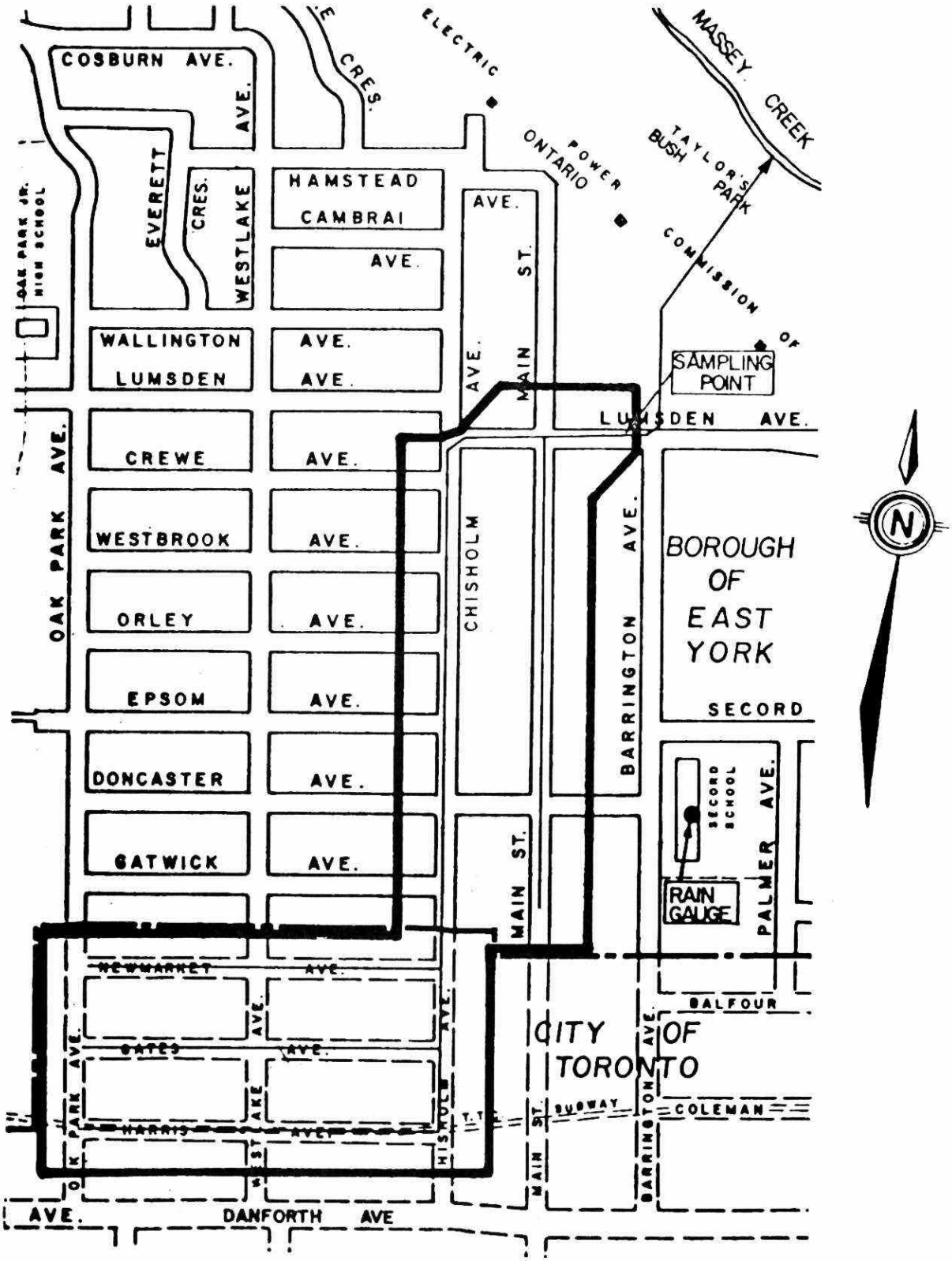

FIGURE 1. BARRINGTON DRAINAGE AREA

3. MATERIALS AND METHODS

3.1 Sample Collection

At both sites, water samples were collected manually in presterilized glass sampling bottles (ca. 170 ml) every five minutes for a period of one hour after the onset of a rainfall event. Four storm events were monitored at the East York Barrington outfall, whereas at the Guelph Woodlawn outfall only one event was examined.

Sediment samples (two at East York and one at Guelph) were collected by hand in sterilized Nalgene wide-mouth jars (ca. 125 ml) on days when no event occurred.

Moore swabs, as modified by Spino [24] and Dutka and Bell [25], were installed in each storm sewer before the start of a rainfall event. Each swab was made of eight double layers of gauze folded to form a 20 x 35 cm pad. The pad was then cut into five 3-5 cm wide strips, which were gathered together at one end to form a 5 cm handle. The handle of the pad was attached to a 30 x 30 cm square of chicken wire; and each strip was spread and separately tied with string to the wire mesh so as to expose maximum surface area to the water. The pad and wire frame were wrapped in Kraft paper and sterilized by autoclaving at 121^{o}C for 15 minutes. In the field, the swab was unwrapped, and suspended by tying one end to a buoy with a one-metre long rope and by attaching (with another rope) the other end to a supporting structure.

Moore swabs were exposed to storm water throughout the entire sampling period during each rainfall event. After exposure, the swabs were retrieved from the sampling site, drained for a few minutes, and placed in large sterile plastic bags.

All Moore swabs, water and sediment samples were transported on ice to the laboratory and were analyzed within 24 hours of collection.

3.2 Microbiological Analyses

In all microbiological analyses where the membrane filtration (MF) technique was employed, Millipore 47 mm, 0.45 µm, white, gridded, ethylene oxide sterilized membrane filters (HAWG 04700) were used. All agar, broth and enrichment media used in microbiological determinations were Bacto brand, supplied by Difco Laboratories, Detroit, Michigan, U.S.A. Exceptions from the above are separately specified.

3.2.1 Pollution indicator bacteria

3.2.1.1 Storm water runoff. Water samples were analyzed for total coliform (TC), fecal coliform (FC) and fecal streptococcus (FS) using the membrane filtration technique. The procedures used for the estimation of these pollution indicator bacteria are described in detail in the Ministry of the Environment's "Handbook of Analytical Methods For Environmental Samples", Volume 2 [26]. Therefore, only a brief account of these methods is given below.

Total coliform densities were determined on m-Endo agar Les plates after incubation at 35 $\pm$ 0.5°C for 22 $\pm$ 2 h. All colonies with a dull to bright metallic, green-gold sheen were considered members of the coliform group. Colony counts were made and the densities were expressed as coliforms per 100 ml sample. All red, pink, blue, white or colourless colonies lacking sheen were considered non-coliforms and were recorded as "background" count per 100 ml sample.

The MacConkey membrane broth plates were used for fecal coliform determinations following incubation (in a floating plastic "cakette") at 44.5 $\pm$ 0.5°C in a circulating waterbath for 20 $\pm$ 1 h. All yellow, yellow-brown, and yellow-green colonies were counted as fecal coliform colonies. Densities were recorded as fecal coliforms per 100 ml sample.

Fecal streptococci were determined using m-enterococcus agar plates incubated at 35 $\pm$ 0.5°C for 48 $\pm$ 3 h. All red, maroon or pink colonies were counted as fecal streptococci. Counts were expressed as fecal streptococci per 100-ml sample.

3.2.1.2 Sediment. The MF technique (as described above) and the most probable number (MPN) technique, following American Public Health Association Standard Methods [27], were used for the enumeration of pollution indicator bacteria in sediments. For both methods, a 1/10 sediment dilution was prepared by mixing 50 g (wet wt.) sediment in 450 ml buffered water dilution blank [26] in a Waring blender for one minute. From this suspension, further 10-fold dilutions were prepared using buffered water as required for various analyses.

TC were determined using a three-dilution, five-tube replication of lactose broth in a standard MPN series. After incubation at 35°C

for 48 h, lactose broth cultures positive for gas were inoculated into brilliant green lactose bile broth tubes and incubated at 35°C for an additional 48 h. All tubes showing acid and gas production were recorded as confirmed TC for the purpose of the MPN computation (MPN/100 ml).

For FC determinations, positive lactose broth cultures were used to inoculate EC broth tubes which were incubated at 44.5 ± 0.5°C for 24 h. Tubes showing growth and gas production were recorded as confirmed FC and were included in the final MPN index of FC.

Fecal streptococci MPN were estimated using a five-tube, three-dilution sodium azide dextrose broth series. Inoculated tubes were incubated at 35°C for 48 h. All cultures showing growth were transferred into ethyl violet azide broth and were then incubated at 35°C for an additional 48 h. Ethyl violet tubes showing turbidity and a purple pellet at the bottom of the tube were recorded as confirmed FS.

3.3.2 Pseudomonas aeruginosa

P. aeruginosa densities in water samples were estimated on mPA agar medium using MF technique as described by Levin and Cabelli [28]. After filtration of appropriate volumes of samples, membranes were transferred to mPA plates and incubated at 41.5 ± 0.5°C for 48 h. All flat colonies (0.8 to 2.2 mm in diameter) with tan, brown, greenish-black centres and pale outer rims were counted, and the densities were expressed as *P. aeruginosa* per 100-ml sample.

P. aeruginosa levels in sediments were enumerated by the MF procedure (as described above) and the MPN technique [27] using a three-dilution, five-tube series of modified Drake's medium 10 [29]. The medium consisted of asparagine, 2.0 g; K_2HPO_4, 1.0 g; $MgSO_4 \cdot 7H_20$, 0.5 g; K_2SO_4, 10.0 g; glycerol, 10.0 g per litre of distilled water. The tubes were incubated at 41.5 ± 0.5°C for 48-96 h and examined under long-wave ultraviolet light. Those tubes showing greenish fluorescent pigment were interpreted as presumptive positive. All presumptive positive tubes were confirmed by streaking on modified Christensen's acetamide agar and skim milk agar. The acetamide agar contained acetamide, 10.0 g; NaCl, 5.0 g; K_2HPO_4, 1.4 g; KH_2PO_4, 0.7 g; $MgSO_4 \cdot 7H_2O$, 1.54 g; phenol red, 0.012 g; agar, 15.0 g per litre of distilled water. The milk agar was prepared by dissolving 100.0 g skim milk (Difco) and 15.0 g agar in two

separate aliquots of 500 ml distilled water. These two mixtures were sterilized separately and were mixed prior to pouring into petri dishes.

Milk agar plates incubated at 35°C for 84 - 96 h, were observed for casein hydrolysis and production of greenish fluorescent pigment. Acetamide agar plates were incubated at 41.5 ± 0.5°C for 48 - 96 h. Orange-red coloration around the colonies was considered as positive confirmation for P. aeruginosa MPN computation.

3.2.3 Coagulase-positive Staphylococci (Staphylococcus aureus)

S. aureus densities in water samples were determined by MF technique using modified Baird-Parker's medium [30, 31]. The medium was prepared by dissolving 63.0 g of Baird-Parker's medium (Oxoid) in 1000 ml distilled water and sterilized by autoclaving at 121°C for 15 minutes. To the medium cooled to 50°C, the following compounds were added aseptically: sulphamezethine (Matheson, Coleman and Bell), 5 mg/100 ml; actidione (Upjohn Company), 150 mg/100 ml; concentrated egg yolk-tellurite emulsion (Oxoid), 5 ml/100 ml. All ingredients were mixed thoroughly and the medium was poured into sterile petri plates which were used within 48 h of preparation.

Following filtration of appropriate volumes of water samples, membrane filters were placed on the modified Baird-Parker's medium plates and incubated at 35 ± 0.5°C for 24 - 48 h. All black, shiny, circular and convex colonies with a 2 - 5 mm clear zone (underneath the membrane filter) were scored as presumptive coagulase-positive staphylococci. All such colonies were streaked on Trypticase soy agar (BBL), incubated at 35 ± 0.5°C for 24 h, and subjected to Gram Stain and coagulase-mannitol tests. The latter test was performed by inoculating tubes containing approximately 3.3 ml coagulase mannitol broth (BBL). For comparison, positive and negative control tubes were also used. All tubes were incubated at 35 ± 0.5°C for 18 - 24 h. Tubes showing coagulation (clot formation) and mannitol fermentation (production of yellow colour) were recorded as positive confirmation for coagulase-positive staphylococci (S. aureus); the densities were expressed as per 100 ml sample.

3.2.4 Salmonella

For the isolation and identification of salmonellae, procedures outlined in "Methods for Microbiological Analysis of Waters, Wastewaters and Sediments" [32] were used with few modifications. The methods used in this study are summarized in Figure 2, and are described in detail in the following sections.

3.2.4.1 Storm water runoff. Storm water samples collected every five minutes during the October 1, 1975 (East York outfall) event were analyzed individually. Water samples collected every five minutes during all other storm events were combined and analyzed as five (A,B,C,D and E) 'composite samples'. The protocol used to pool water samples is described in Section 4.

Water samples in variable aliquots (ranging from 10 to 400 ml) were filtered through 47-mm sterilized fiberglass prefilters (Sartorius SM 13400, British Drug House, Toronto, Ontario). The filters were aseptically placed in corresponding equal volumes of tetrathionate and selenite broths prewarmed to 41.5°C. These enrichment broths were incubated at 41.5°C for 20 ± 2 h, and a loopful of each broth was streaked on Brilliant Green (BG) agar and on Xylose Lysine Deoxycholate (XLD) agar plates. All plates were incubated at 41.5°C for 20 ± 2 h. After incubation, colonies appearing to be salmonellae* were streaked on MacConkey agar plates and also inoculated into O-nitrophenol β-D-galactopyranoside (BDH) - phenylalanine agar (ONPG-PA) tubes, which in turn were incubated overnight (20 ± 2 h) at 35°C. Lactose negative (MacConkey agar) and ONPG-PA negative colonies were inoculated into tubes of Motility Sulfide Medium (MSM), Triple Sugar Iron (TSI) agar and H broth. Presumptive Salmonella colonies growing on MacConkey agar plates were used as the source of inoculum for these tests. After incubation at 35°C for 20 ± 2 h, reactions on TSI and MSM were recorded. The MSM cultures were then overlayed with 1.0 ml of urea broth and incubated at 35°C for approximately 6 h to determine urease activity. A 3.0 ml aliquot of H broth was added to an equal volume of 0.6 N formyl saline, and a 2.0 ml aliquot was tested for indole production with Kovac's

* Pink-opaque colonies on BG and pink-red, shiny black centred colonies on XLD.

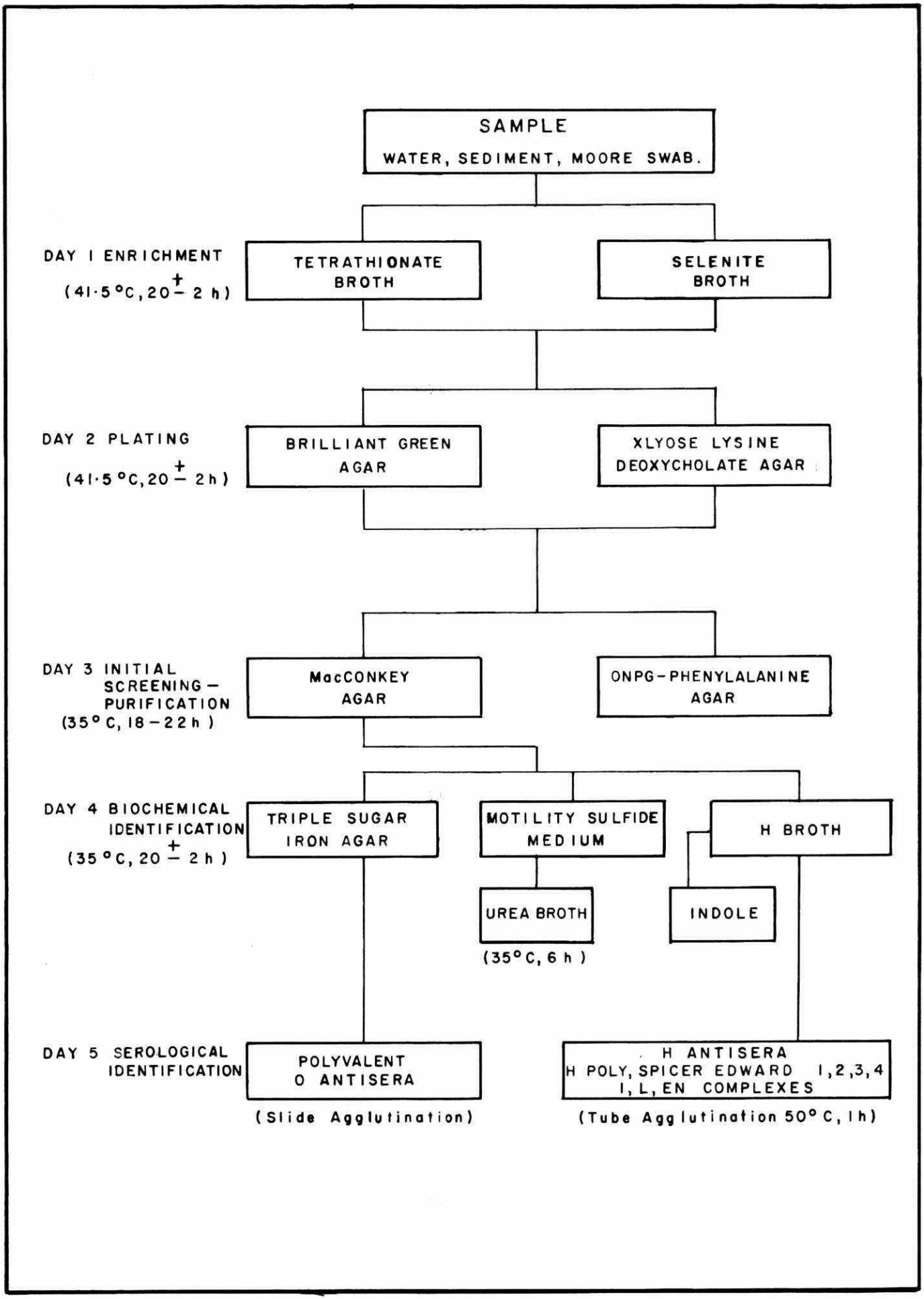

FIGURE 2: SCHEME FOR SALMONELLA ISOLATION AND IDENTIFICATION

reagent. Cultures giving reactions typical for Salmonella in these media were biochemically and serologically identified by the methods of Edwards and Ewing [33].

Cultures that produced alkaline slants, acid butt, and gas, with or without H_2S in TSI, and which exhibited motility and H_2S production in MSM, and which were also urease negative, were subjected to serological testing. All cultures were first tested for Salmonella O (somatic) antigens by slide agglutination with Salmonella polyvalent O antisera-using growth on TSI slants. Cultures showing a positive reaction were then tested for Salmonella H (flagellar) antigens by multiple tube agglutination test. Approximately 0.5 ml of formalin-treated H broth was added to 0.5 ml of 1:200 dilution of the following Salmonella H antisera: H poly, Spicer Edward 1:4, EN Complex, I Complex and L Complex. All tubes for H antigens were incubated for 1 h at 50°C in a water bath. Cultures confirming to this stage as Salmonella were sent to the Ontario Ministry of Health, Enteric Reference Centre, Rexdale, Ontario, for confirmation and identification of Salmonella serotypes.

3.2.4.2 Moore swab. The five strips of each Moore swab were aseptically separated with sterile scissors and forceps. Three strips were transferred aseptically to separate 450-ml capacity wide-mouthed glass bottles containing 300 ml of tetrathionate broth prewarmed to 41.5°C. The remaining two strips were each placed aseptically in 300 ml of prewarmed (at 41.5°C) selenite broth. The incubation and further processing of Moore swab enrichment broths for Salmonella isolation was similar to the methods described above for water samples.

3.2.4.3 Sediment. The membrane filter and direct inoculation techniques were used for the isolation of Salmonella from sediments. One-ml aliquots of ten-fold sediment dilutions (preparation method described under the pollution indicator bacteria section) were filtered through membrane filters. The membranes were aseptically transferred to prewarmed (at 41.5°C) 10-ml volumes of tetrathionate and selenite enrichment broths. In addition, 1, 10, 50 or 100 ml aliquots of 1/10 sediment suspensions were directly inoculated into appropriate volumes

(1:10, 10 and 50:50, 100:100) of tetrathionate and selenite broths prewarmed to 41.5°C.

Twenty-five gram aliquots of one sediment sample (from East York) were directly placed into 300-ml volumes of prewarmed tetrathionate and selenite broths. All enrichment broths were incubated and further analyzed for Salmonella isolation following the procedures outlined above for water samples.

3.2.5 Total fungi (molds and yeasts)

Fungal densities both in water and sediments were estimated by the spread plate (SP) technique using Streptomycin Terramycin Malt Extract Agar (STMEA) medium. This medium, which contained 30.0 g Malt extract, 5.0 g peptone and 15.0 g agar, was sterilized by autoclaving at 121°C for 15 minutes. Membrane filter sterilized solutions of streptomycin (Sigma Chemical Company, Saint Louis, Missouri) and terramycin (Pfizer Company Ltd., Montreal, Quebec) were separately added to the cooled autoclaved medium to obtain a final concentration (of each antibiotic) of 70 µg/ml.

Sample and/or sample dilutions of water and sediment (prepared as described in Section 3.2.1) were plated in one-ml aliquots onto STMEA plates. The plates were predried, with covers removed, in a filtered air laminar flow hood at room temperature for 1 h. For plating, the Fisher Rota-Plate Inoculating Turntable (Fisher Scientific Company, Toronto, Ontario) was used to obtain a uniform distribution of the sample. The inoculated plates were allowed to dry on the laboratory bench, inverted and then incubated (in a plastic cakette) at 20°C for seven days. The developing colonies of both yeast and molds (total fungi) were counted with the aid of a Quebec Colony Counter. Fungal densities were expressed as colony-forming units (CFU) per 100-ml water sample and CFU per gram sediment sample.

4. RESULTS

4.1 Barrington Avenue (East York), Toronto

4.1.1 Storm water runoff

Microbiological data obtained from storm water samples collected during four storm events are presented in Appendix I-IV. Due to the uniqueness and diverse nature of all storm events, their results are described individually.

Storm of October 1, 1975. A total of 2.3 mm rain fell during the storm, which lasted for 30 minutes. Sampling was initiated 15 minutes after the onset of the storm and continued for 40 minutes after the end of the storm (Appendix I).

The densities of pollution indicator bacteria in storm water samples are shown in Figure 3. Total coliform (TC) levels showed an oscillating pattern of distribution. Initial counts (380 000/100 ml) exhibited a generally rising trend with the increasing time of sampling for up to 20 minutes. The densities then decreased slightly only to peak again in the 35-minute sample. Thereafter, counts declined to around 530 000/100 ml in the 45 to 55-minute samples. Fecal coliform (FC) densities ranged from 120 000 to 420 000/100 ml and showed a distribution pattern similar to that of TC. The highest FC count was recorded in the 35-minute sample, while in the 0 and 55-minute samples the densities were 120 000 and 140 000/100 ml, respectively. Fecal streptococcus (FS) levels varied from 230 000 to 620 000/100 ml, and unlike TC and FC, showed a relatively uniform distribution pattern. The maximum FS count was noted in the 10-minute sample, whereas the lowest count was obtained in the 45-minute sample. In all samples, the FS densities were generally much higher than those of FC.

The initial low counts of total fungi (3 000 000/100 ml) also exhibited a generally rising trend to reach the maximum level (9 000 000/100 ml) in the 15-minute sample (Figure 3). Thereafter, fungal densities declined steadily and the lowest counts were observed in the 55-minute sample.

S. aureus was detected in only seven samples and the densities were generally low, ranging from 12 to 40/100 ml (Appendix I).

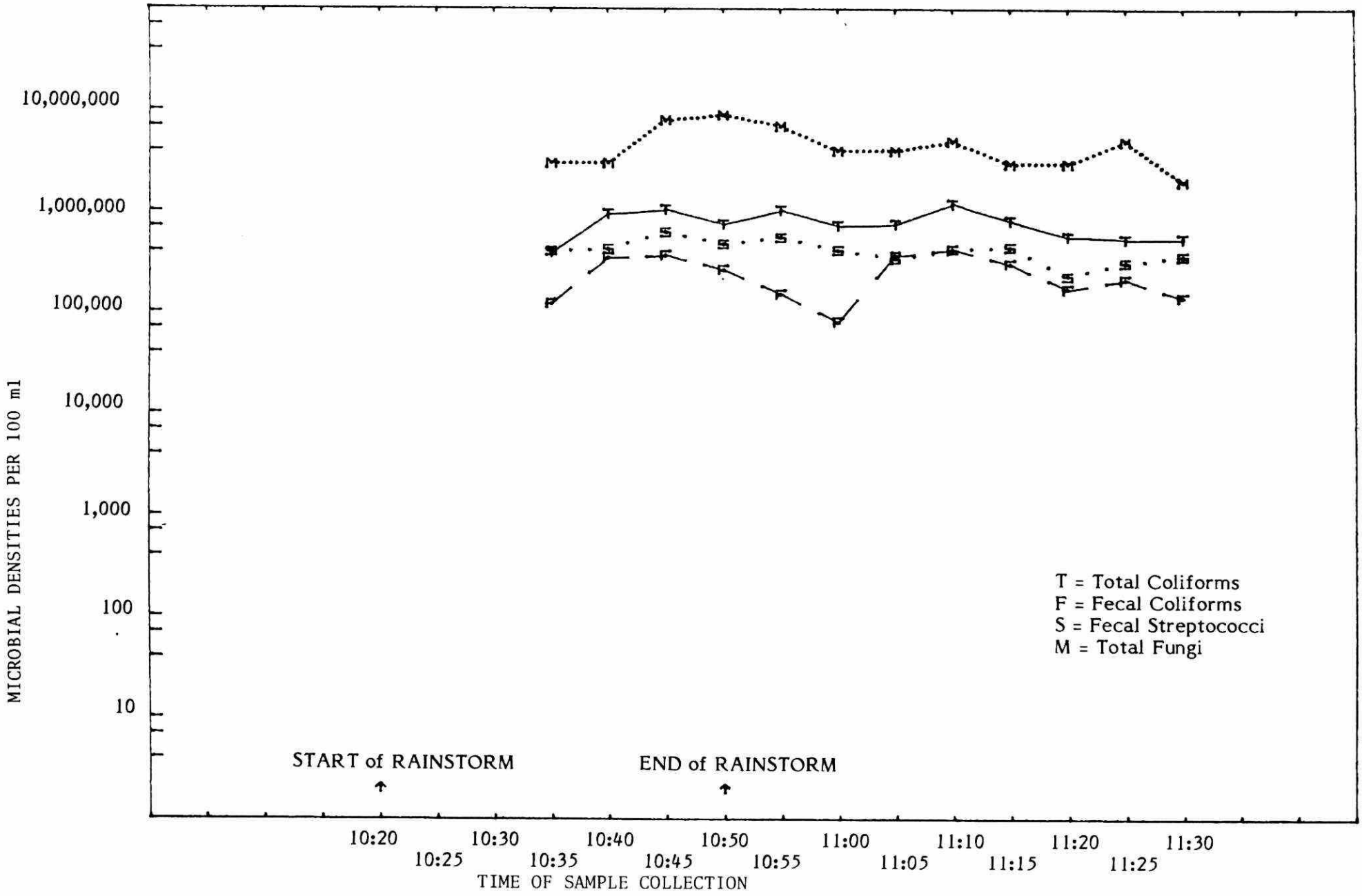

FIGURE 3. DISTRIBUTION AND LEVELS OF POLLUTION INDICATOR BACTERIA AND TOTAL FUNGI IN STORM WATER RUNOFFS, OCTOBER 1, 1975, BARRINGTON AVENUE (EAST YORK), TORONTO

Salmonellae were isolated only from the 15, 20, 25 and 30-minute samples (Appendix I). Two Salmonella serotypes, including S. typhimurium var. copenhagen and S. saint-paul, were isolated from as little as 10 ml of the water samples using tetrathionate-brilliant green media combination. No salmonellae were isolated from the Moore swab during this storm event.

Storm of November 7, 1975. Only 1.0 mm rainfall accumulated during the storm event, which was of 50 minutes duration. Sampling began 15 minutes after the initiation of the storm and was completed 25 minutes after the end of the storm event (Appendix II).

The levels and the distribution of the TC, FC and FS in storm water samples are given in Figure 4. The peak populations of these bacteria were observed in the first (0 minute) sample which was in fact collected 15 minutes after the commencement of the storm. Thereafter, total and fecal coliform densities decreased and increased with no consistent pattern of distribution. TC counts ranged from 240 000 to 790 000/100 ml, and FC levels ranged from 10 000 to 550 000/100 ml (Appendix II). After the initial peak, FS densities declined steadily but showed a relatively uniform pattern of distribution. The minimum and maximum values for FS were 10 000 and 240 000/100 ml, respectively. Furthermore, with few exceptions, FS densities were consistently higher than FC in storm water runoff samples.

The maximum levels of fungi (12 000 000/100 ml) were found in the 0 minute (first) sample. In general, fungal densities decreased with the increasing time of sampling and the lowest count, 3 500 000/100 ml, was observed in the last (60-minute) sample (Appendix II and Figure 4).

Low levels (1 to 10/100 ml) of S. aureus were recorded in all storm water samples (Appendix II).

During this event, salmonellae were detected in all (A to E) composite storm water samples (Appendix II). Twelve isolates of Salmonella tennessee were obtained from these samples. Two isolates of the same Salmonella serotype were found in the Moore swab. Of the various media used, tetrathionate-brilliant green and selenite-brilliant green yielded five and four S. tennessee isolates, respectively.

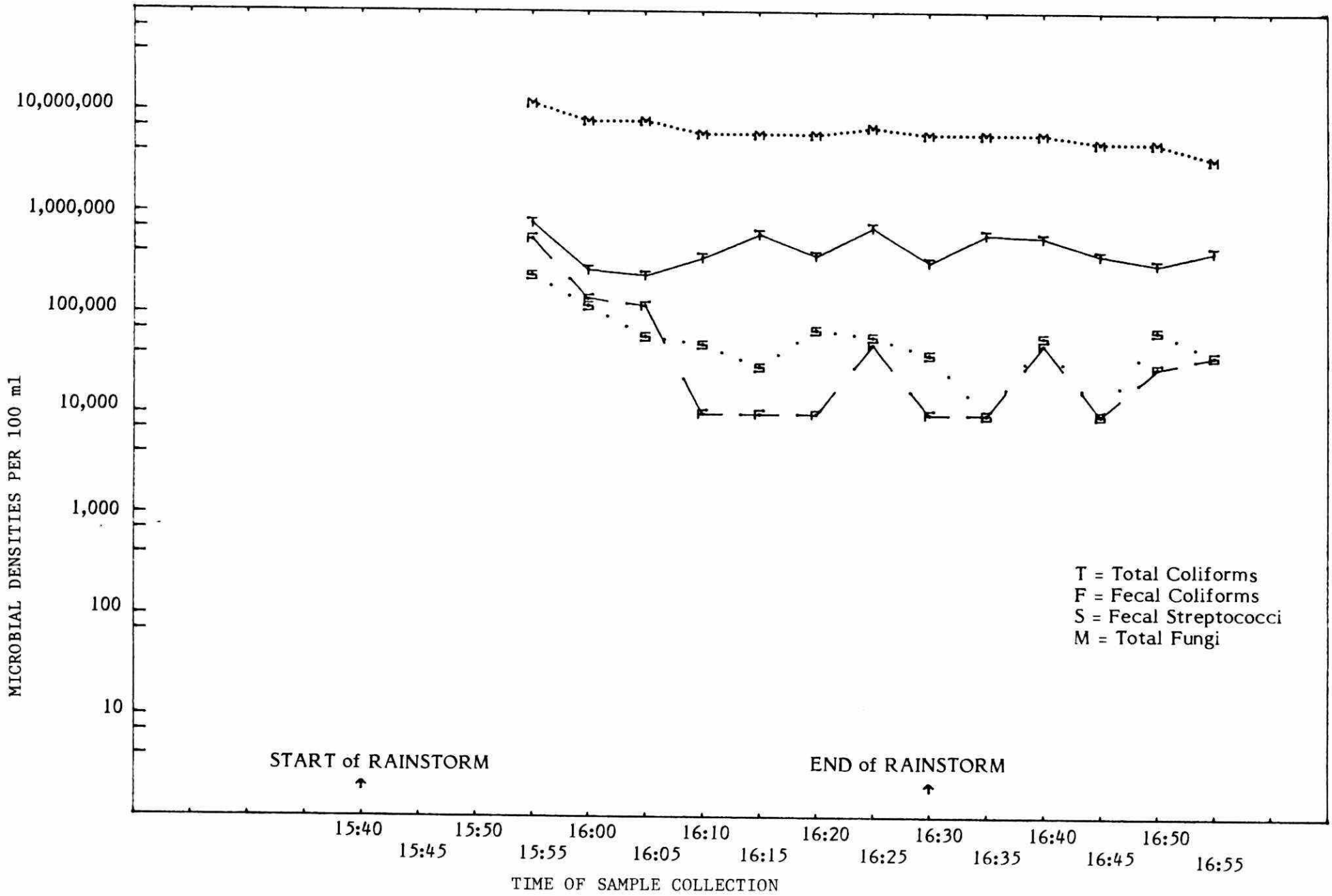

FIGURE 4. DISTRIBUTION AND LEVELS OF POLLUTION INDICATOR BACTERIA AND TOTAL FUNGI IN STORM WATER RUNOFFS, NOVEMBER 7, 1975, BARRINGTON AVENUE (EAST YORK), TORONTO

Storm of December 30, 1975. During this storm event, which was of considerably longer duration (735 minutes), approximately 10.9 mm rainfall occurred. The first sample was obtained at 9:45 a.m. (390 minutes after the start of the storm), while the last sample was collected at 10:40 a.m. The storm event, however, continued till 3:30 p.m. (Appendix III).

The levels of indicator bacteria in all storm water samples are presented in Figure 5. The densities of TC, FC and FS were generally low in 0 to 40-minute samples. However, elevated levels of these organisms were observed in samples collected at the 45 to 55-minute marks. Furthermore, the counts of all three indicator bacteria groups showed enormous variation. For example, TC values ranged from 7000 to 53 000/100 ml, FC from 1300 to 22 400/100 ml, and FS 4700 to 33 000/100 ml.

Total fungal densities ranged from 400 000 to 900 000/100 ml. The highest counts were observed in the 30 and 50-minute samples, while the lowest level was recorded in the last (55-minute) sample (Appendix III, and Figure 5).

S. aureus was found in low numbers (10 to 20/100 ml) in only six of all the storm water samples. P. aeruginosa was detected in all the samples, with densities ranging from 130 to 630/100 ml (Appendix III). No salmonellae were isolated from either the Moore swab or any of the composite storm water samples during the course of this storm event.

Storm of January 26, 1976. The storm lasted 495 minutes and a total of 12.2 mm rain fell during this event. The sampling, however, was initiated at 9:15 a.m. (approximately 55 minutes after the end of the storm) and continued till 10:05 a.m. (Appendix IV). Since all samples were collected well after the rainfall had ended, they were not "typical" storm water runoff samples and were mainly composed of snow-melt runoff.

The numbers and distribution of pollution indicator bacteria are illustrated in Figure 6. The densities of TC, FC and FS varied considerably throughout the entire sampling period and no consistent pattern of their distribution was established. As an example, TC levels (per 100 ml) ranged from 23 000 to 35 000 in 0 to 10-minute samples, from 14 000 to 33 000 in 15 to 35-minute samples, peaked (61 000) at the 40-minute mark, and varied from 18 000 to 31 000 in 45 to 55-minute

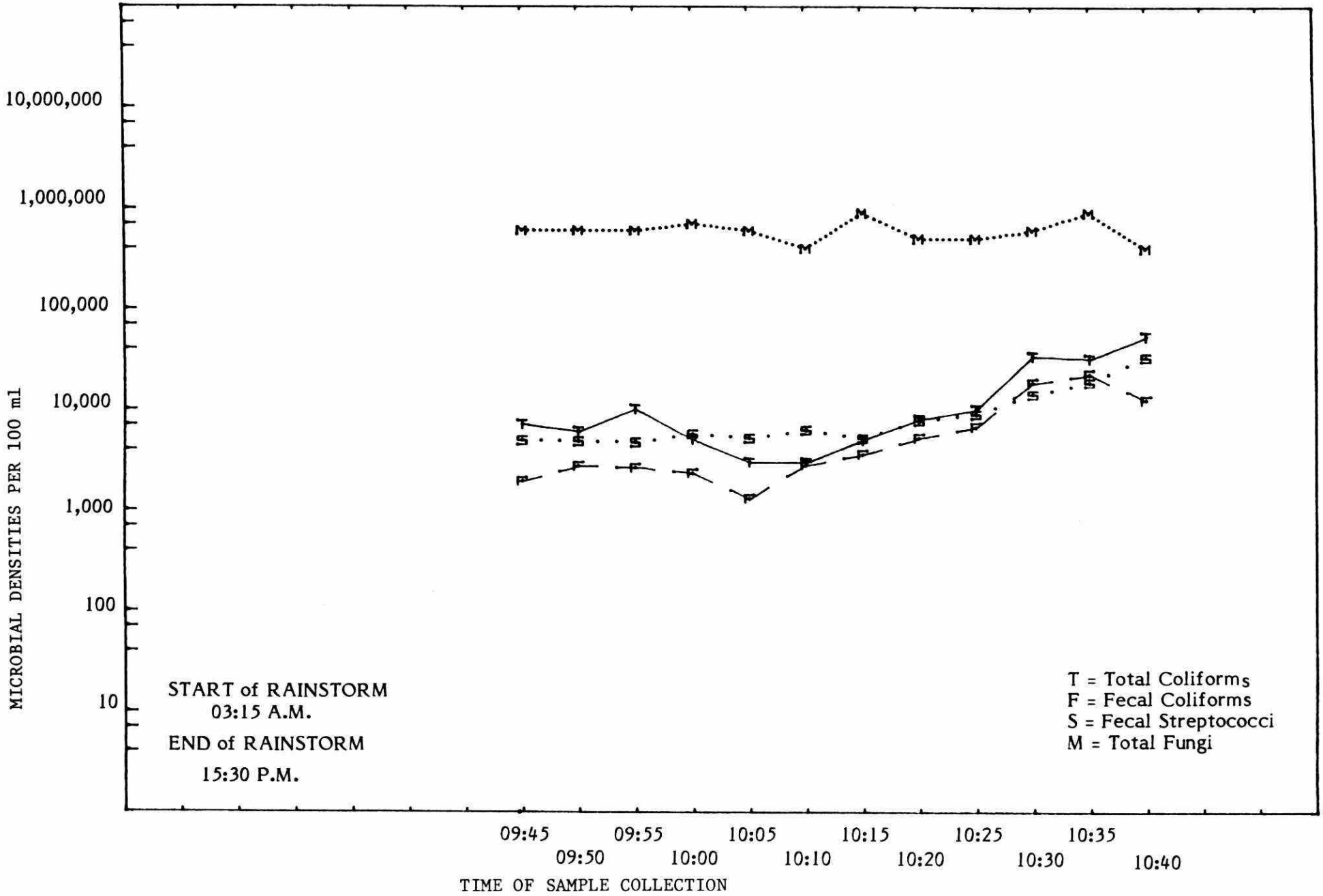

FIGURE 5. DISTRIBUTION AND LEVELS OF POLLUTION INDICATOR BACTERIA AND TOTAL FUNGI IN STORM WATER RUNOFFS, DECEMBER 30, 1975, BARRINGTON AVENUE (EAST YORK), TORONTO

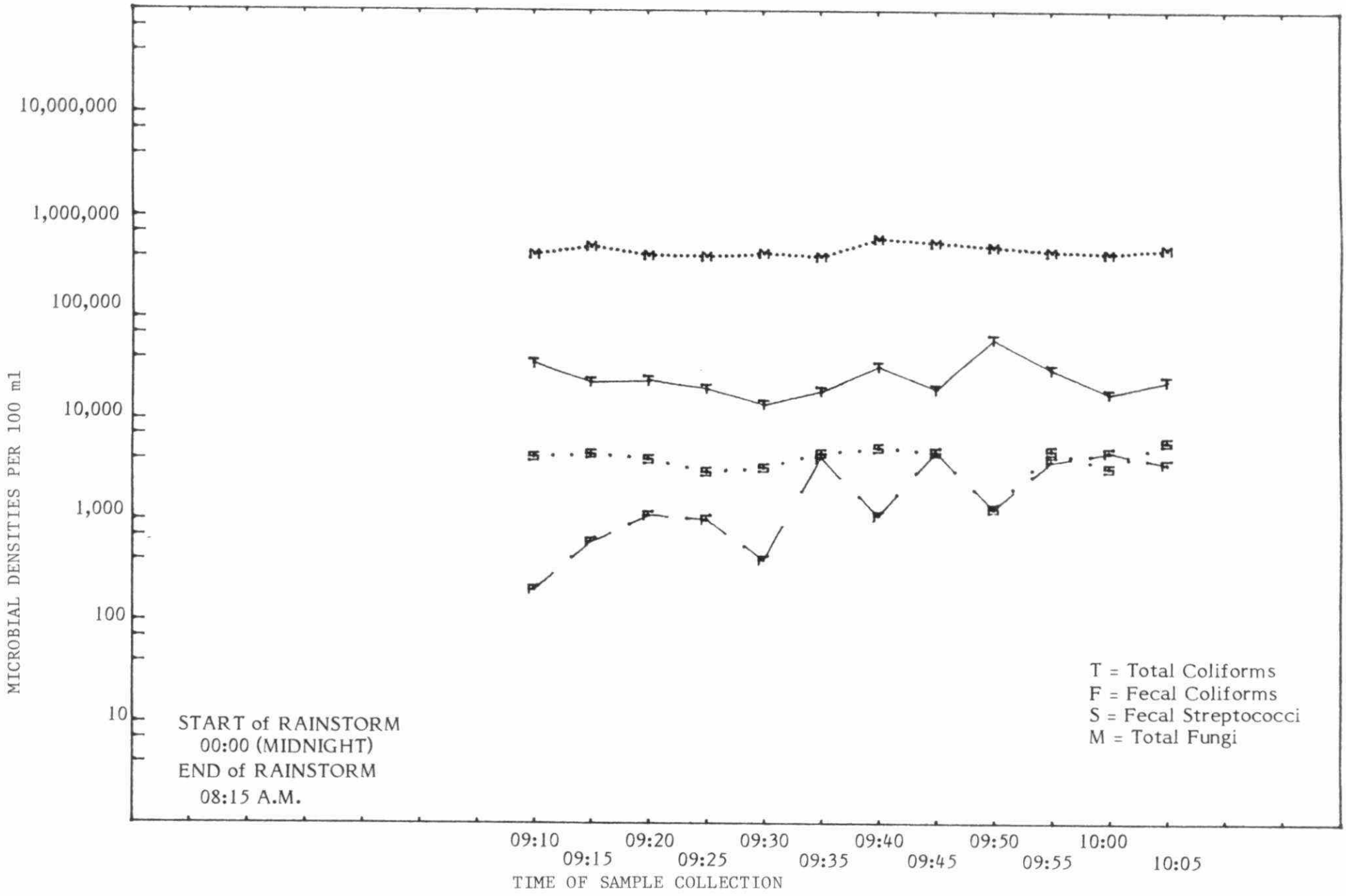

FIGURE 6. DISTRIBUTION AND LEVELS OF POLLUTION INDICATOR BACTERIA AND TOTAL FUNGI IN STORM WATER RUNOFFS, JANUARY 26, 1976, BARRINGTON AVENUE (EAST YORK), TORONTO

samples. Likewise, FC numbers (per 100 ml) ranged from a low of 200 in the 0-minute sample to 47 000 in the 35 to 45-minute samples. FS counts ranged from 3000 to 6000/100 ml, but exhibited less sample to sample variation compared to the total and fecal coliform counts.

The densities of total fungi varied between 410 000 to 6 000 000/100 ml and showed little sample to sample variation. The maximum count was recorded in the 30-minute sample, while the minimum count was observed in the 10-minute sample (Appendix IV and Figure 6).

As summarized in Appendix IV, S. aureus was detected in all but two samples. The levels ranged from 10 to 100/100 ml. P. aeruginosa was isolated in low levels (10 to 20/100 ml) from only 50% of the samples. Salmonella typhimurium was isolated from composite storm water samples A (one isolate), D (two isolates), and E (three isolates). However, no salmonellae were detected in the Moore swab. Three of the six S. typhimurium isolates were obtained from the selenite-XLD media combination.

4.1.2 Sediment

Microbiological data obtained from the two sediment samples collected during dry weather conditions are given in Table 1. The densities of indicator bacteria were high, ranging from 10^4 to 10^8 per 100 ml. With one exception, the MF method produced higher TC, FC and FS counts than the MPN technique. As determined by the MF technique, the TC counts were 2 700 000 and 177 000,000; FC densities were 130 000 and 180 000; and FS levels were 90 000 and 190 000 in the two samples examined.

P. aeruginosa densities could not be satisfactorily determined using either the MPN or MF technique because appropriate sediment dilutions were not analyzed. In both samples, fungal densities were appreciably high, i.e. 46 000 and 660 000 per gram sediment.

As shown in Table 2, no salmonellae were isolated from the September 29 sediment sample using either MF or direct inoculation (DI) techniques and the various enrichment-plating media combinations. However, in the November 4 sample, Salmonella typhimurium var. copenhagen was isolated from the tetrathionate-XLD combination when 25 grams sediment were directly inoculated into the enrichment medium. The other media

TABLE I. MICROBIAL DENSITIES IN SEDIMENT SAMPLES COLLECTED FROM STORM SEWERS AT BARRINGTON AVENUE (EAST YORK), TORONTO AND WOODLAWN ROAD, GUELPH

Date	Site and Sample Collection Time	BACTERIAL PARAMETERS PER 100 ML								
		Total Coliform		Fecal Coliform		Fecal Streptococcus		Pseudomonas aeruginosa		Total Fungi[1]
		MF[2]	MPN[3]	MF	MPN	MF	MPN	MF	MPN	MF
September 29, 1975	Barrington Avenue 10:00	177 000 000	1 600 000	130 000	17 000	90 000	110 000	< 1 000	< 100	46 000
October 21, 1975	Woodlawn Road 14:00	260 000	1 600 000	20 000	49 000	10 000	79 000	< 1 000	< 100	33 000
November 4, 1975	Barrington Avenue 10:30	2 700 000	1 300 000	180 000	110 000	190 000	22 000	< 1 000	< 100	660 000

1 - Total Fungi CFU/G (Wet Weight Sediment).

2 - Membrane Filtration Technique.

3 - Most Probable Number Technique.

TABLE 2. ISOLATION OF SALMONELLA FROM SEDIMENT SAMPLES COLLECTED FROM STORM SEWERS AT BARRINGTON AVENUE (EAST YORK), TORONTO AND WOODLAWN ROAD, GUELPH

Date	Site and Sample Collection Time	SALMONELLA	
		Isolation Pathway *	Serotype
September 29, 1975	Barrington Avenue 10:00	MF (0.1, 0.01, 0.001, 0.0001 ml via serial sediment dilution) - TB, TX, SB, SX	NEG **
		DI (1,10, 50 ml of 1/10 sediment dilution) - TB, TX, SB, SX	NEG
October 21, 1975	Woodlawn Road 14:00	MF (0.1, 0.01, 0.001, 0.0001 via serial sediment dilution) - TB, TX, SB, SX	NEG
		DI (1, 10, 50 ml of 1/10 sediment dilution) - TB, TX, SB, SX	NEG
November 4, 1975	Barrington Avenue 10:30	DI (1, 50, 100 ml of 1/10 sediment dilution) - TB, TX, SB, SX	NEG NEG
		DI (25 g wet weight sediment) - TX	Salmonella typhimurium var. copenhagen
		- TB, SB, SX	NEG

* MF: Membrane Filtration Technique, DI: Direct Inoculation Technique.

Enrichment Broths, Temperature, Incubation: T = Tetrathionate, 41.5°C, 20 ± 2 h; S = Selenite, 41.5°C, 20 ± 2 h.

Plating Agars, Temperature, Incubation: B = Brilliant Green Agar, 41.5°C, 20 ± 2 h; X = XLD Agar, 41.5°C, 20 ± 2 h.

** NEG: Negative.

combinations did not yield any Salmonella serotypes with this method of inoculation.

4.2 Woodlawn Road, Guelph

4.2.1 Storm water runoff

Storm of November 12, 1975. During this event no specific rainfall information was collected. The microbiological data obtained from the anlysis of storm water samples of this event are presented in Appendix V and Figure 7.

The TC levels (per 100 ml) varied considerably during the entire sampling period. For instance, in the first (0-minute) sample the TC count was 3800; thereafter, densities increased and ranged from 6200 to 10 200 in samples collected between the 5 and 45-minute marks. The peak (17 700) TC densities were observed in the 50-minute sample. The 55 and 60-minute samples yielded TC counts of 14 600 and 13 600, respectively. FC densities were generally low and varied from 220 to 630/100 ml exhibiting little sample to sample variation. FS levels showed an irregular pattern of distribution and the counts varied from 4 000 (60-minute sample) to 17 000 (30-minute sample) per 100 ml. Furthermore, in all samples, the FS densities were significantly (at least 20 times) higher than the FC levels.

Fungal densities (Appendix V and Figure 7) were generally low (140 000/100 ml) in the 0 to 10-minute samples. With one exception, fungal counts showed a relatively uniform distribution pattern in all of the other samples and the values ranged from 200 000 to 280 000/100 ml. The maximum level was recorded in the 30-minute sample.

S. aureus (1/100 ml) was found only in the 5 and 10-minute samples. P. aeruginosa was detected in all samples. In the 0 to 30-minute samples the densities were generally low (1 to 8/100 ml). However, slightly higher counts (28 to 32/100 ml) were recorded in the 50 to 60-minute samples (Appendix V).

No salmonellae were found in any of the storm water samples. Three isolates of Salmonella haardt were obtained from the Moore swab exposed to the storm water for 60 minutes during this event. Two of these isolates were obtained from the tetrathionate-BG combination, and selenite-BG yielded the third isolate.

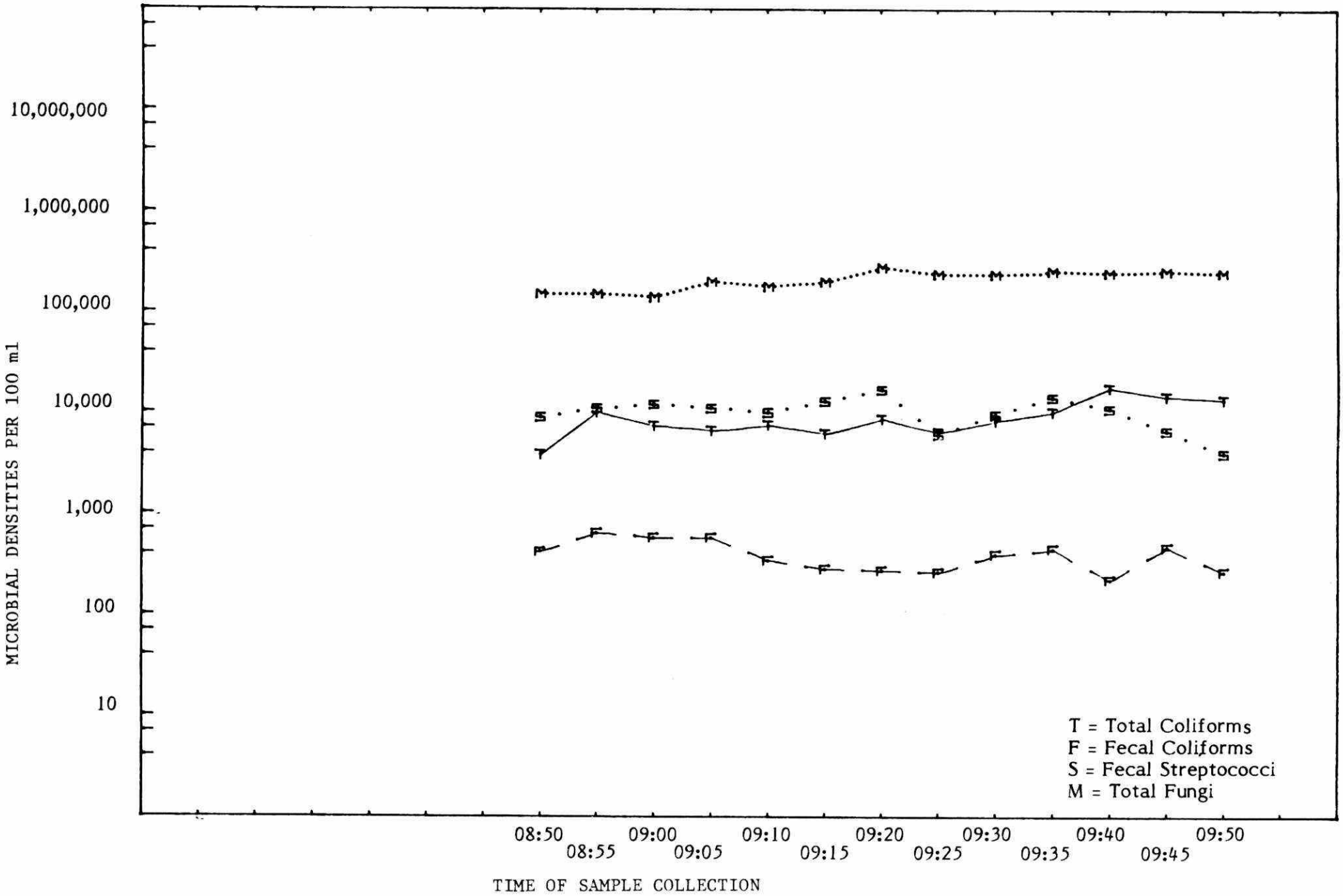

FIGURE 7. DISTRIBUTION AND LEVELS OF POLLUTION INDICATOR BACTERIA AND TOTAL FUNGI IN STORM WATER RUNOFFS, NOVEMBER 12, 1975, WOODLAWN ROAD, GUELPH

4.2.2 Sediment

Microbiological data obtained from the only sediment sample collected during dry weather conditions are shown in Table 1. The levels of all pollution indicator bacteria examined were generally high and varied from 10^4 to 10^6 per 100 ml. The TC, FC and FS densities as determined by the MPN method (1 600 000, 49 000 and 79 000, respectively) were greater than the corresponding values obtained using the MF technique.

As inappropriate sediment dilutions were analyzed, no suitable counts of *P. aeruginosa* could be determined. In this sample, fungal densities were 33 000 per gram sediment. In addition, no salmonellae were detected in this sample using either the DI or MF procedures in conjunction with different combinations of enrichment and plating media (Table 2).

5. DISCUSSION

5.1 Summary and Synthesis of all Microbiological Data

During this investigation, storm water samples were collected manually at least 15 minutes after the onset of a storm event. In one instance, although the storm event started at midnight, samples were collected in the morning, approximately one hour after the rainfall had ended. As a result, strictly speaking, no "zero" minute sample was collected during any of the storms monitored. The collection of such a sample was extremely difficult since, after the start of a storm, the field staff had to drive a substantial distance (requiring 10-20 minutes travelling time) to reach the sampling site. In any event, for the purpose of this investigation, the first sample collected was considered as the "zero" minute sample; thereafter, the sampling process continued for one hour during which additional samples were collected at five-minute intervals.

In addition to sample collection problems, the monitored rainstorms varied in intensity, duration and in the amount of total rainfall. As a result, no relationships or trends were evident with respect to the occurrence of maximum microbial populations in storm water runoffs. For example, the peak microbial populations occurred either at the initial stages, middle or the tail-end of the sampling period of different storms monitored.

Although some variation was observed, in general, the initial counts (from 0 to 10-minute samples) of pollution indicator bacteria and total fungi were either at about the same level or slightly higher than the counts from 15 to 60-minute samples. This indicates that any effect of "initial flushing" on microbiological quality of the discharges was minimal during an individual storm.

All storm water samples collected during the study period, from the two separate storm sewers, contained significant quantities of pollution indicator bacteria. However, no typical levels or patterns of distribution of these organisms could be established for individual storms. At the Barrington Avenue (East York, Toronto) site, the densities of TC varied from 3000 to 1 190 000/100 ml. The TC geometric

means for all the samples ranged from 9330 to 730 000/100 ml (Figure 8). Fecal coliform levels ranged from 200 to 550 000/100 ml, while FC geometric means ranged from 1460 to 216 000/100 ml (Figure 8). Fecal streptococcus densities were generally equal to or greater than the FC levels. The minimum and maximum FS counts were 3000 and 620 000 per 100 ml, respectively. Geometric mean fecal streptococcus densities ranged from 4520 to 402 000/100 ml (Figure 8).

At the Woodlawn Road (Guelph) site, the densities of pollution indicator bacteria were lower than those obtained at the Toronto site. As summarized in Appendix V and Figure 8, the TC levels varied from 3800 to 17 700 with a geometric mean of 8560 per 100 ml. Fecal coliform counts ranged from 220 to 630 per 100 ml with a geometric mean of 370. Fecal streptococcus densities were greater than FC levels and ranged from 6100 to 17 000 per 100 ml, the geometric mean value being 9730.

Several other investigators [2,5,6,10] reported TC, FC and FS densities in storm waters from separate storm sewer systems similar to those found in the present study. As an example, Weibel et al [2] found that in 50% of all storm water samples collected from a separate storm sewer, the levels of TC, FC and FS were greater than 58 000, 10 900 and 20 500 per 100 ml, respectively. Furthermore, Geldreich et al [10] reported seasonal variation (autumn densities higher than winter) in the populations of indicator bacteria in storm waters collected from suburban areas. Benzie and Courchaine [6] found that the levels of coliforms were lower in storm water runoffs during winter and early spring than for the warmer weather periods. Similar results were obtained in the present study as the TC, FC and FS densities were considerably greater (at the Toronto site) in samples obtained during October-November than those collected in December-January period.

Fungal population estimates varied between 400 000 and 12 000 000 per 100 ml in storm waters collected from the Toronto site. The geometric mean values for all samples ranged from 460 000 to 6 240 000 (Figure 8). In discharges from the Guelph storm sewer, the minimum and maximum fungal densities were 140 000 and 280 000 with a geometric mean of 210 000 per 100 ml (Figure 8).

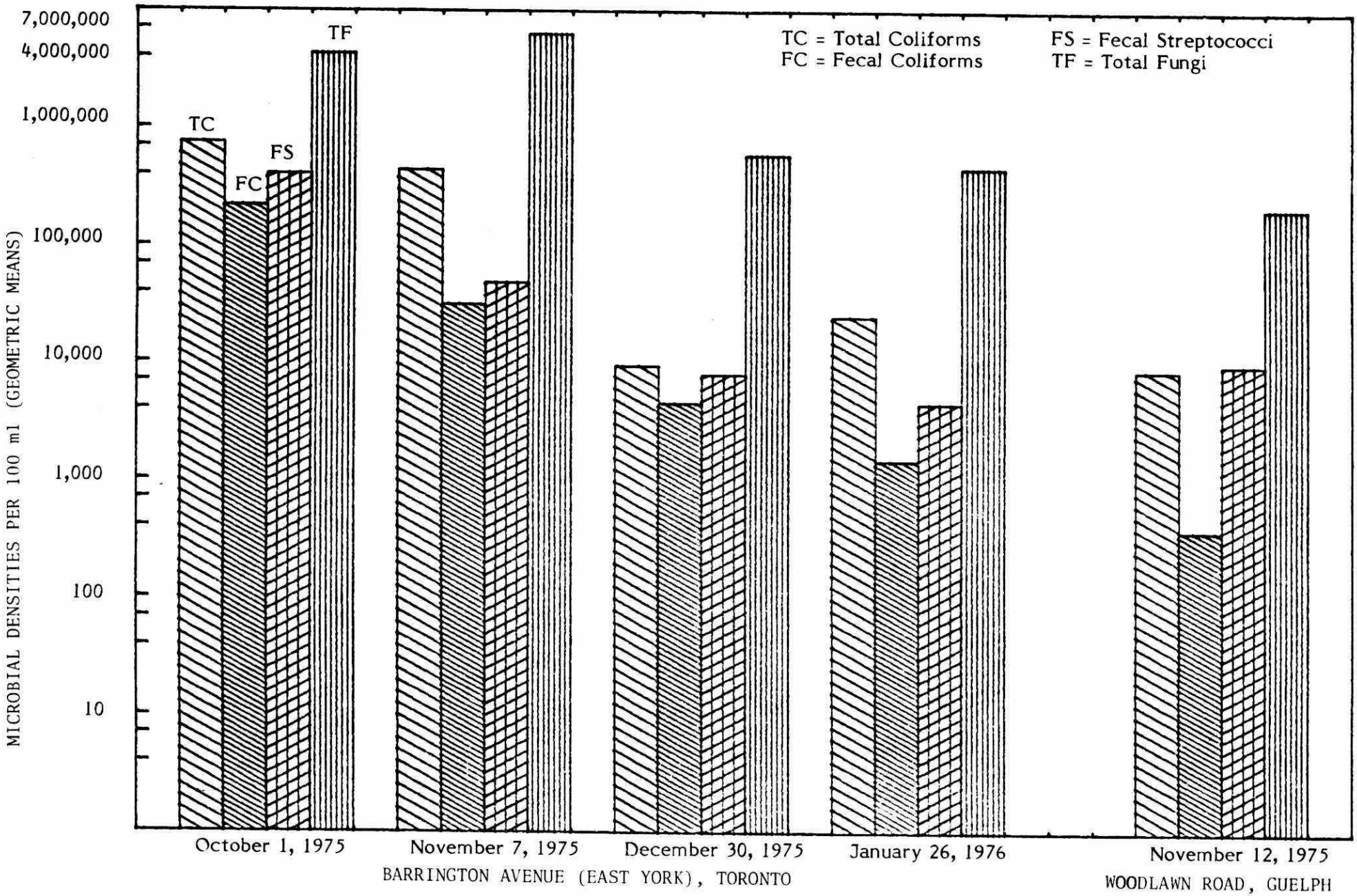

FIGURE 8. MICROBIAL POPULATIONS IN DISCHARGES FROM FIVE STORM EVENTS AT THE TWO URBAN SEPARATE STORM SEWERS

With respect to opportunistic pathogenic bacteria, S. aureus was infrequently isolated from storm waters at both sites. The densities were generally low and, with few exceptions, ranged from 1 to 40 per 100 ml. P. aeruginosa was found in only 50% of the samples. It was detected in all samples examined and the densities (10 to 630/100 ml) at the Toronto site were greater than the Guelph storm sewer (1 and 32 per 100 ml).

During this investigation, 28 isolates of Salmonella were detected from storm waters at both the Guelph and Toronto storm sewers (Table 3). These isolates belonged to four different serotypes including S. saint-paul, S. haardt, S. tennessee and S. typhimurium. Most of the isolates (about 90%) were obtained from the Toronto storm sewer, where salmonellae were detected in as little as 10 ml of storm water sample (Appendix I). Salmonellae were also isolated from 50, 100 and 300 ml aliquots of either separate or composite storm water samples and from a Moore swab (Appendix I, II and III). At the Guelph site, Salmonella was found only in the Moore swab and not in the sediment sample.

As with the pollution indicator bacteria, no definite pattern of occurrence and distribution of salmonellae was evident in this study. These organisms were found, though at different time intervals, in all samples collected during the 60-minute sampling period. This indicates that pathogenic bacteria like Salmonella, are probably discharged throughout the entire span of a storm event.

Evans et al [9] reported the presence of Salmonella in a storm water sample collected from an urban separate storm sewer which contained significant quantities of fecal pollution indicator bacteria. They estimated that one isolate of Salmonella was detected for every 100 fecal coliforms in that sample. In this study no relationships were evident between the incidence of salmonellae and the levels and peaks of indicator bacteria in storm waters examined. It must be pointed out, however, that it is generally assumed that the chances of Salmonella isolation are greater from those samples which contained large numbers of fecal coliforms than from those with low fecal coliform densities [34,35,36]. The relationships between pathogenic microorganisms and indicator organisms have also been reviewed recently by several workers in the

TABLE 3. SUMMARY OF SALMONELLA ISOLATION FROM STORMWATER RUNOFF AND SEDIMENT SAMPLES COLLECTED FROM THE TWO SEPARATE STORM SEWERS

SITE	SALMONELLA SEROTYPE	NUMBER OF ISOLATES
Barrington Avenue (East York, Toronto)	Sediment	
	Salmonella typhimurium var. copenhagen	1
	Stormwater Runoff	
	S. saint-paul	1
	S. tennessee	14
	S. typhimurium	6
	S. typhimurium var. copenhagen	3
Woodlawn Road (Guelph)	Stormwater Runoff (Moore Swab)	
	S. haardt	3
TOTAL:		28

"Proceedings of Workshop on Microorganisms in Urban Stormwater" [37] and in a comprehensive report on "Microorganisms in Urban Stormwater" [58].

Sediment samples collected during dry weather conditions from these two storm sewers yielded large quantities of fecal indicator bacteria. Their levels were either equal to or greater than those in storm water runoffs. Fungal densities were also substantially higher in sediments. Salmonella was found in only one sediment sample obtained from the Toronto storm sewer. The serotype S. typhimurium var. copenhagen was also fund in storm waters at this site.

5.2 Sources of Microbial Pollution in Storm Water Runoff and Associated Potential Health Hazards

It may be assumed that there is no apparent source of contamination with domestic waste discharges in either the Guelph or Toronto residential storm sewers as they are both separate storm water systems. The microbiological data from these storm sewers, however, demonstrated that storm water discharges contained substantial "fecal" pollution. The levels of TC, FC and FS were strikingly high throughout the sampling period of 60 minutes, and many times reached densities found in untreated domestic sanitary discharges. The high concentration of indicator bacteria was accompanied by the presence of certain pathogenic and potentially pathogenic bacteria in these storm water runoffs.

Geldreich and Kenner [38] proposed that the sources of fecal bacterial pollution may be differentiated by using a fecal coliform to fecal streptococcus (FC:FS) ratio. It was suggested that if the FC:FS ratio is greater than 4.0, the source is likely of human origin; while if the ratio is less than 0.7 then the source is probably of non-human origin. The FC:FS ratios between 0.7 and 4.0 have been considered as "indeterminate" as to source; they may be from mixed, old or atypical sources. Although this concept has received general acceptance and has been widely used, several workers [38,39,40,41,42,43] emphasized that the FC:FS ratio must be employed with some degree of caution since it is time dependent. They pointed out that, once discharged into receiving waters, the differential die-off rates of these organisms and diverse environmental factors may alter their interrelationship to such an extent as to render the FC:FS ratio of limited or no significance in determining the source of bacterial contamination.

The results obtained in this study indicate that a relationship between fecal coliforms and fecal streptococci does exist within certain limits of interpretation. At the Guelph site, fecal contamination is primarily of non-human origin as fecal streptococcus densities were significantly greater than fecal coliforms. The data from the Toronto storm sewer are rather inconclusive; however, FS levels, which are generally equal to or greater than FC counts, would indicate that bacterial contamination is predominately of non-human origin. It is possible that, at this site, a portion of fecal contamination may have originated from the unknown entry of sanitary discharges through illegal cross-connections. Previous investigators [2,5,6,9,10,38,58] reported similar findings and demonstrated that fecal pollution in the separate storm sewer systems is predominately of non-human origin.

Fecal contamination in these two separate storm sewer systems, situated in residential areas, appears to be derived initially from feces deposited by pets*, particularly cats and dogs, rodents and birds. In addition, runoff from soil previously contaminated with animal waste may contribute to fluctuating levels of fecal contamination to drainage water. During periods of rainfall, some of the indicator bacteria associated with a variety of vegetation [44] may also enter the storm water as part of overland-wash. Furthermore, it has been suggested that both coliforms and non-coliforms proliferate (characterized as after-growths) in sediments and soil and as such may be of little sanitary significance [17,45]. Nevertheless, the strikingly high levels of microbial populations observed in sediments in this study do indicate that storm sewer sediments constitute a reservoir of indicator bacteria, as well as pathogens that may eventually find their way into storm water runoffs.

The remarkably high concentration of fecal pollution indicator bacteria in discharges from these two residential urban separate storm sewers indicate possible health hazards. Water bodies receiving untreated

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* According to a conservative estimate made by the Toronto Humane Society "there are about 44,000 dogs and 48,000 cats in the city of Toronto". In Canada, a cross-country survey in 1975 showed "that in cities of 100,000 or more 25% of the homes had one or more dogs and that 24% of the homes had one or more cats" (The Globe and Mail, Toronto, May 11, 1977).

storm waters are not suitable for bathing, swimming and general recreation because such discharges are potential causes of human and animal infections.

P. aeruginosa (an opportunistic pathogen) has become a common cause of nosocomial infections [46,47] and is regarded as a major etiological agent of otitis externa among swimmers [47,48]. The presence of P. aeruginosa in storm waters at both sites also constitutes an elevated risk to public health.

The detection of salmonellae in both storm water and sediments in this study further substantiates the existence of potential health hazards in storm water runoffs. Of the Salmonella serotypes isolated, S. typhimurium and S. saint-paul were among the 10 most common serotypes isolated from humans in Denmark and in the United States [49]. S. typhimurium was also one of the 13 strains most frequently isolated from both diseased and healthy farm animals [49]. Moreover, all four serotypes obtained in this study have also been isolated from humans in Canada during the past several years. For example, in Ontario in 1975 (Personal Communications from Dr. A. Evans, Community Health Protection Branch, Ontario Ministry of Health, Toronto), S. typhimurium and S. thyphimurium var. copenhagen were most frequently isolated; while the incidence of S. saint-paul, S. tennessee and S. haardt was less common. S. typhimurium var. copenhagen was involved in one outbreak which accounted for 46 cases of illness.

During this investigation, substantially high populations of fungi (including both molds and yeasts) were found in sediments and storm water discharges at the two sites examined. Although the procedures used in this study did not delineate the saprophytic and pathogenic fungi, their presence in large quantities demonstrated that storm water runoffs may contribute yet another group of potential pathogens to the recipient waters. Bergen and Wagner-Merner [50] recently reported the occurrence of potentially pathogenic fungi at several beaches in the Tampa Bay area.

5.3 Urban Storm Water Runoff Pollution Control

In recent years urban storm water runoff has been recognized as a significant source of a wide variety of pollutants to surface waters.

The information provided in this report further substantiates that urban storm water discharges are a major source of intermittent microbial pollution to receiving water. The results of various other studies and general awareness of harmful effects from urban storm water runoffs have motivated the development and implementation of programs to control water pollution from such nonpoint sources. All of the methods proposed and designed to control pollution from storm water runoffs include different types of storage of storm waters and their subsequent treatment by various means. Several investigators [11,15,20,23,52-57] have provided comprehensive reviews of different methods employed in planning, management and control of urban nonpoint source pollution.

Furthermore, in order to prevent and control microbial pollution in storm water runoffs, certain remedial measures should be applied at sources of such contamination. These may include: (i) pet population control, (ii) efficient and effective removal of animal waste from streets, (iii) sanitation and effective garbage control to discourage rodent habitation of storm sewers, and (iv) improved maintenance of storm sewers to avoid excessive accumulation of sediments which act as reservoirs of microbial contamination. The remedial measures should also include education and awareness programs to better inform the general public about the existence, significance, sources and control of urban pollution from nonpoint sources.

5.4 Comments on Methodologies Used in This Study

5.4.1 Sample collection

Problems were encountered in manual sample collection of storm waters, particularly in obtaining "zero" minute samples since the technician had to drive to the site after the initiation of a storm event. In addition, during those events which started during the night, samples were collected in the morning when the storm had already been in progress for several hours.

An alternative would be automatic sampling which was not attempted in the present study. However, samples collected by automatic means are suitable for microbiological examination only if they are collected in sterile refrigerated containers. Such an automatic sampler is not available at the present time.

In future studies, manual sample collection should be carried out by a local resident who can immediately reach the site following the onset of the storm. Of course, this arrangement will require some initial training of such a person for the collection of microbiological samples.

5.4.2 Pollution indicator bacteria

The MF techniques and procedures used for the isolation and enumeration of TC, FC and FS from storm water runoff samples were found to be satisfactory and provided meaningful estimates of populations of these organisms. Since problems were encountered in analyzing sediments by the MF technique, the most probable number (MPN) technique should be used in future studies for the enumeration of pollution indicator bacteria.

5.4.3 Pseudomonas aeruginosa

The use of MF technique and the mPA medium were satisfactory for the determination of P. aeruginosa densities in water samples. The levels in sediments, however, should be ascertained by the MPN procedure using three to five dilution/tube series.

5.4.4 Coagulase-positive Staphylococci (Staphylococcus aureus)

The method used for the determination of S. aureus levels in water samples proved to be unsatisfactory. The procedure is lengthy, tedious, requires several confirmatory steps and as such is not appropriate for routine analysis. In addition, problems were encountered during the enumeration step due to the presence of false positive and false negative colonies. As a result the medium and procedure are not suitable for S. aureus isolation from environmental samples. Further work is required to either modify this medium or develop a new method for the enumeration of S. aureus from natural waters.

5.4.5 Total fungi (molds and yeasts)

The spread plate technique and STMEA medium were found to be suitable for the determination of fungal populations both in water and sediments. The MF technique can also be used for the enumeration of fungi in natural waters as described previously [32].

5.5 Salmonella

Since storm water runoffs contained remarkably high levels of microbial populations, salmonellae were isolated from as little as 10 ml sample aliquots. The maximum sample volume which yielded Salmonella was 300 ml. However, for the detection of salmonellae in waters from a less polluted environment, the use of larger volumes is recommended. This can be achieved by filtering sample aliquots ranging from 500 to 1000 ml through three or four prefilters (similar to those used in this study), which then can be placed in the enrichment broth. The use of Moore swabs was also satisfactory for Salmonella isolation and their use, where possible, is highly recommended for any future studies of this nature.

Although the scheme used here for the detection of Salmonella requires five days for positive identification, it was reliable and very productive. Once the MacConkey agar and ONPG-PA agar screening test were completed (on the third day), only a small number of non-salmonellae were eliminated on the basis of subsequent biochemical tests. With respect to the Salmonella isolation pathway, 24 h enrichment at 41.5°C was fairly productive; however, extended (48 h) incubation may enhance recovery of Salmonella. Whereas both tetrathionate and selenite broths provided almost equal numbers (15 and 13, respectively) of Salmonella isolates, the BG plating medium yielded 19 isolates compared to nine on XLD agar. The tetrathionate-BG combination was the most productive and produced 11 (39.3%) isolates; this was followed by selenite-BG with 8 (28.6%) isolates. In comparison, the tetrathionate-XLD and selenite-XLD combinations were less productive and yielded four (14.3%) and five (17.8%) isolates, respectively.

The use of sediment suspension-dilution was unsuccessful for the detection of salmonellae in sediments. Salmonella was isolated only once when sediments (25 g) were inoculated directly into the enrichment broth. This procedure is highly recommended for any future studies of salmonellae isolation from sediments.

The method used in this study provided only qualitative determination of salmonellae; in future, attempts should be made to develop procedures for quantitative isolation of Salmonella. Furthermore, additional research should be conducted, using several other enrichment

broths and plating agars, to select one most productive combination for Salmonella isolation from natural waters. The use of only one enrichment broth and plating medium will decrease the test cost and workload considerably, and may also permit extended (48 h) enrichment.

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A P P E N D I C E S

APPENDIX I. LEVLES AND DISTRIBUTION OF MICROORGANISMS IN STORMWATER RUNOFF FROM BARRINGTON AVENUE (EAST YORK) STORM SEWER, TORONTO

A. POLLUTION INDICATOR BACTERIA, PSEUDOMONAS AERUGINOSA, STAPHYLOCOCCUS AUREUS, TOTAL FUNGI

STORM			SAMPLE COLLECTION		BACTERIAL PARAMETERS PER 100 ML					
Date	Duration (Minutes)	Amount of Rainfall	Time	Accumulative Time (Minutes)	Total Coliform	Fecal Coliform	Fecal Streptococcus	Pseudomonas aeruginosa	Staphylococcus aureus	TOTAL FUNGI (CFU†/100 ML)
October 1, 1975	10:20 - 10:50	2.3 mm	10:35	0	380,000	120,000	400,000	NA*	< 10	3,000,000
			10:40	5	930,000	340,000	420,000	NA	< 10	3,000,000
	(30)		10:45	10	1,030,000	360,000	620,000	NA	< 10	8,000,000
			10:50	15	740,000	260,000	470,000	NA	< 10	9,000,000
			10:55	20	1,020,000	150,000	550,000	NA	30	7,000,000
			11:00	25	720,000	80,000	410,000	NA	20	4,000,000
			11:05	30	750,000	360,000	340,000	NA	OBS**	4,000,000
			11:10	35	1,190,000	420,000	420,000	NA	40	5,000,000
			11:15	40	810,000	300,000	440,000	NA	40	3,000,000
			11:20	45	560,000	170,000	230,000	NA	20	3,000,000
			11:25	50	530,000	210,000	310,000	NA	16	5,000,000
			11:30	55	540,000	140,000	360,000	NA	12	2,000,000

* NA: Not Analyzed.

** OBS: Obscured.

† CFU: Colony- forming units.

APPENDIX I. (Continued)

B. SALMONELLA

SAMPLE COLLECTION			SALMONELLA	
Time	Accumulative Time (Minutes)	Composite Sample	Isolation Pathway *	Serotype
10:35	0	-	10, 50, 100 - TB, TX, SB, SX	NEG**
10:40	5	-	——— " ———	NEG
10:45	10	-	——— " ———	NEG
10:50	15	-	10 - TB	Salmonella saint-paul
			50, 100 - TB, TX	NEG
			10, 50, 100 - SB, SX	NEG
10:55	20	-	10 - TB	S. typhimurium var. copenhagen
			50, 100 - TB, TX	NEG
			10, 50, 100 - SB, SX	NEG
11:00	25		10 - TB	S. typhimurium var. copenhagen
			50, 100 - TB, TX	NEG
			10, 50, 100 - SB, SX	NEG
11:05	30	-	50 - TB	S. typhimurium var. copenhagen
			10, 100 - TB, TX	NEG
			10, 50, 100 - SB, SX	NEG
11:10	35	-	10, 50, 100 - TB, TX, SB, SX	NEG
11:15	40	-	——— " ———	NEG
11:20	45	-	——— " ———	NEG
11:25	50	-	——— " ———	NEG
11:30	55	-	——— " ———	NEG
11:35	60	Moore Swab	Strips 1, 2, 3 - TB, TX	NEG
			4, 5, - SB, SX	NEG

* Sample Volume Analyzed: 10, 50, 100 ml.

Enrichment Broths, Temperature, Incubation: T = Tetrathionate, 41.5°C, 20 ± 2 h; S = Selenite, 41.5°C, 20 ± 2 h.

Plating Agars, Temperature, Incubation: B = Brilliant Green Agar, 41.5°C, 20 ± 2 h; X = XLD Agar, 41.5°C, 20 ± h.

** NEG: Negative.

APPENDIX II. LEVELS AND DISTRIBUTION OF MICROORGANISMS IN STORMWATER RUNOFF FROM BARRINGTON AVENUE (EAST YORK) STORM SEWER, TORONTO

A. POLLUTION INDICATOR BACTERIA, PSEUDOMONAS AERUGINOSA, STAPHYLOCOCCUS AUREUS, TOTAL FUNGI

STORM			SAMPLE COLLECTION		BACTERIAL PARAMETERS PER 100 ML					
Date	Duration (Minutes)	Amount of Rainfall	Time	Accumulative Time (Minutes)	Total Coliform	Fecal Coliform	Fecal Streptococcus	Pseudomonas aeruginosa	Staphylococcus aureus	TOTAL FUNGI (CFU/100 ML)
November 7, 1975	15:40 - 16:30 (50)	1.0 mm	15:55	0	790,000	550,000	240,000	NA*	1	12,000,000
			16:00	5	270,000	140,000	120,000	NA	4	8,000,000
			16:05	10	240,000	120,000	60,000	NA	1	8,000,000
			16:10	15	360,000	10,000	50,000	NA	4	6,000,000
			16:15	20	620,000	10,000	30,000	NA	10	6,000,000
			16:20	25	380,000	10,000	70,000	NA	4	6,000,000
			16:25	30	720,000	50,000	60,000	NA	4	7,000,000
			16:30	35	330,000	10,000	40,000	NA	4	6,000,000
			16:35	40	620,000	10,000	10,000	NA	4	6,000,000
			16:40	45	580,000	50,000	60,000	NA	4	6,000,000
			16:45	50	390,000	10,000	10,000	NA	4	5,000,000
			16:50	55	320,000	30,000	70,000	NA	4	5,000,000
			16:55	60	430,000	40,000	40,000	NA	1	3,500,000

* NA: Not Analyzed.

APPENDIX II. (Continued)

B. SALMONELLA

SAMPLE COLLECTION			SALMONELLA	
Time	Accumulative Time (Minutes)	Composite Sample	Isolation Pathway *	Serotype
15:55	0	A	100 - TB	Salmonella tennessee
16:00	5		100 - SB	S tennessee
16:05	10		100 - TX, SX	NEG **
			50, 300 - TB, TX, SB, SX	NEG
16:10	15	B	100 - SB	S. tennessee
16:15	20		100 - TB, TX, SX	NEG
			50, 150 - TB, TX, SB, SX	NEG
16:20	25	C	100 - TX	S. tennessee
16:25	30		150 - TX	S. tennessee
			100, 150 - TB, SB, SX	NEG
			50 - TB, TX, SB, SX	NEG
16:30	35	D	100 - SB	S. tennessee
16:35	40		300 - TB	S. tennessee
16:40	45		300 - SX	S. tennessee
			100 - TB, TX, SX	NEG
			300 - TX, SB	NEG
			50 - TB, TX, SB, SX	NEG
16:45	50	E	300 - TB	S. tennessee
16:50	55		300 - TX	S. tennessee
16:55	60		300 - SB	S. tennessee
			300 - SX	S. tennessee
			50, 100 - TB, TX, SB, SX	NEG
16:55	60	Moore Swab	Strips 1, 2, 3 - TB	S. tennessee
			- TX	NEG
			4, 5, - SB	S. tennessee
			- SX	NEG

* Sample Volume Analyzed: 50, 100, 150, 300 ml.

Enrichment Broths, Temperature, Incubation: T = Tetrathionate, 41.5°C, 20 ± 2 h; S = Selenite, 41.5°C, 20 ± 2 h.

Plating Agars, Temperature, Incubation: B = Brilliant Green Agar, 41.5°C, 20 ± 2 h; X = XLD Agar, 41.5°C, 20 ± 2 h.

** NEG: Negative.

APPENDIX III. LEVELS AND DISTRIBUTION OF MICROORGANISMS IN STORMWATER RUNOFF FROM BARRINGTON AVENUE (EAST YORK) STORM SEWER, TORONTO

A. POLLUTION INDICATOR BACTERIA, PSEUDOMONAS AERUGINOSA, STAPHYLOCOCCUS AUREUS, TOTAL FUNGI

STORM			SAMPLE COLLECTION		BACTERIAL PARAMETERS PER 100 ML					
Date	Duration (Minutes)	Amount of Rainfall	Time	Accumulative Time (Minutes)	Total Coliform	Fecal Coliform	Fecal Streptococcus	Pseudomonas aeruginosa	Staphylococcus areua	TOTAL FUNGI (CFU/100 ML)
December 30, 1975	3:15 -	10.9 mm	9:45	0	7,000	1,900	4,900	330	0	600,000
	15:30*		9:50	5	6,000	2,700	4,800	190	10	600,000
	(735)		9:55	10	10,000	2,600	4,700	270	10	600,000
			10:00	15	5,000	2,300	5,300	130	10	700,000
			10:05	20	3,000	1,300	5,200	290	20	600,000
			10:10	25	3,000	2,800	6,300	290	20	400,000
			10:15	30	5,000	3,600	5,200	240	0	900,000
			10:20	35	8,000	5,200	7,800	630	20	500,000
			10:25	40	10,000	6,700	9,000	350	0	500,000
			10:30	45	34,000	18,500	14,200	520	0	600,000
			10:35	50	32,000	22,400	18,700	320	0	900,000
			10:40	55	53,000	12,400	33,000	330	0	400,000

* Samples were taken during the most intense period of rainfall, and 2.5 mm rain fell during the sampling period.

APPENDIX III. (Continued)

B. SALMONELLA

SAMPLE COLLECTION			SALMONELLA	
Time	Accumulative Time (Minutes)	Composite Sample	Isolation Pathway *	Serotype
9:45	0	A	50, 100, 300 - TB, TX, SB, SX	NEG **
9:50	5			
9:55	10			
10:00	15	B	50, 100, 300 - TB, TX, SB, SX	NEG
10:05	20			
10:10	25	C	50, 100, 150 - TB, TX, SB, SX	NEG
10:15	30			
10:20	35	D	50, 100, 150 - TB, TX, SB, SX	NEG
10:25	40			
10:30	45	E	50, 100, 300 - TB, TX, SB, SX	NEG
10:35	50			
10:40	55			
10:45	60	Moore Swab	Strips 1, 2, 3 - TB, TX	NEG
			4, 5 - SB, SX	NEG

* Sample Volume Analyzed: 50, 100, 150, 300 ml.

Enrichment Broths, Temperature, Incubation: T = Tetrathionate, 41.5°C, 20 ± 2 h; S = Selenite, 41.5°C, 20 ± 2 h.

Plating Agars, Temperature, Incubation: B = Brilliant Green Agar, 41.5°C, 20 ± 2 h; X = XLD Agar, 41.5°C, 20 ± 2 h.

** NEG: Negative.

APPENDIX IV. LEVELS AND DISTRIBUTION OF MICROORGANISMS IN STORMWATER RUNOFF FROM BARRINGTON AVENUE (EAST YORK) STORM SEWER, TORONTO

A. POLLUTION INDICATOR BACTERIA, PSEUDOMONAS AERUGINOSA, STAPHYLOCOCCUS AUREUS, TOTAL FUNGI

STORM			SAMPLE COLLECTION		BACTERIAL PARAMETERS PER 100 ML					
Date	Duration (Minutes)	Amount of Rainfall	Time	Accumulative Time (Minutes)	Total Coliform	Fecal Coliform	Fecal Streptococcus	Pseudomonas aeruginosa	Staphylococcus aureus	TOTAL FUNGI (CFU/100 ML)
January 26, 1976	00:00 -	12.2 mm	9:10	0	35,000	200	4,200	10	10	420,000
	8:15*		9:15	5	23,000	600	4,500	0	20	500,000
	(495)		9:20	10	24,000	1,100	4,000	0	10	410,000
			9:25	15	20,000	1,000	3,000	0	20	400,000
			9:30	20	14,000	400	3,300	0	10	430,000
			9:35	25	19,000	4,100	4,600	20	100	400,000
			9:40	30	33,000	1,100	5,200	10	0	600,000
			9:45	35	20,000	4,700	6,600	0	100	550,000
			9:50	40	61,000	1,300	6,300	10	0	500,000
			9:55	45	31,000	3,800	4,900	0	0	450,000
			10:00	50	18,000	4,700	3,300	10	100	430,000
			10:05	55	24,000	3,600	6,000	10	10	480,000

* Samples were taken after rainfall ended and were mainly composed of melting-snow runoff.

APPENDIX IV. (Continued)

B. SALMONELLA

SAMPLE COLLECTION			SALMONELLA	
Time	Accumulative Time (Minutes)	Composite Sample	Isolation Pathway *	Serotype
9:10	0	A	50 - TB	Salmonella typhimurium
9:15	5		50 - TX, SB, SX	NEG **
9:20	10		100, 300 - TB, TX, SB, SX	NEG
9:25	15	B	50, 100, 150 - TB, TX, SB, SX	NEG
9:30	20			
9:35	25	C	50, 100, 150 - TB, TX, SB, SX	NEG
9:40	30			
9:45	35	D	50 - SB	S. typhimurium
9:50	40		50 - SX	S. typhimurium
			50 - TB, TX	NEG
			100, 150, TB, TX, SB, SX	NEG
9:55	45	E	50 - SX	S. typhimurium
10:00	50		100 - SB	S. typhimurium
10:05	55		100 - SX	S. typhimurium
			50 - TB, TX, SB	NEG
			100 - TB, TX	NEG
			300 - TB, TX, SB, SX	NEG
10:10	60	Moore Swab	Strips 1, 2, 3 - TB, TX	NEG
			4, 5 - SB, SX	NEG

* Sample Volume Analyzed: 50, 100, 300 ml.

Enrichment Broths, Temperature, Incubation: T = Tetrathionate, 41.5°C, 20 ± 2 h; S = Selenite, 41.5°C, 20 ± 2 h.

Plating Agars, Temperature, Incubation: B = Brilliant Green Agar, 41.5°C, 20 ± 2 h; X = XLD Agar, 41.5°C, 20 ± 2 h.

** NEG: Negative.

APPENDIX V. LEVELS AND DISTRIBUTION OF MICROORGANISMS IN STORMWATER RUNOFF FROM WOODLAWN ROAD STORM SEWER, GUELPH

A. POLLUTION INDICATOR BACTERIA, PSEUDOMONAS AERUGINOSA, STAPHYLOCOCCUS AUREUS, TOTAL FUNGI

STORM			SAMPLE COLLECTION		BACTERIAL PARAMETERS PER 100 ML					
Date	Duration (Minutes)	Amount of Rainfall	Time	Accumulative Time (Minutes)	Total Coliform	Fecal Coliform	Fecal Streptococcus	Pseudomonas aeruginosa	Staphylococcus areus	TOTAL FUNGI (CFU/100 ML)
November 12, 1975	NA*	NA*	8:50	0	3,800	410	8,900	4	0	150,000
			8:55	5	9,900	630	11,000	8	1	150,000
			9:00	10	7,300	570	12,000	4	1	140,000
			9:05	15	6,600	570	11,000	1	0	200,000
			9:10	20	7,500	340	10,000	4	0	180,000
			9:15	25	6,200	280	13,000	4	0	200,000
			9:20	30	8,600	270	17,000	1	0	280,000
			9:25	35	6,400	260	6,100	24	0	240,000
			9:30	40	8,300	390	9,600	4	0	240,000
			9:35	45	10,200	440	14,200	20	0	260,000
			9:40	50	17,700	220	11,000	28	0	250,000
			9:45	55	14,600	460	6,700	32	0	260,000
			9:50	60	13,600	264	4,000	28	0	250,000

* NA: Not Available.

APPENDIX V. (Continued)

B. SALMONELLA

SAMPLE COLLECTION			SALMONELLA	
Time	Accumulative Time (Minutes)	Composite Sample	Isolation Pathway *	Serotype
8:50	0	A	400 - TB, TX, SB, SX	NEG **
8:55	5			
9:00	10			
9:05	15	B	250 - TB, TX, SB, SX	NEG
9:10	20			
9:15	25	C	200 - TB, TX, SB, SX	NEG
9:20	30			
9:25	35	D	250 - TB, TX, SB, SX	NEG
9:30	40			
9:35	45			
9:40	50	E	350 - TB, TX, SB, SX	NEG
9:45	55			
9:50	60			
9:50	60	Moore Swab	Strips 1 - TB, TX	NEG
			2 - TB	Salmonella haardt
			3 - TB	S. haardt
			2, 3 - TX	NEG
			4 - SB, SX	NEG
			5 - SB	S. haardt
			5 - SX	NEG

* Sample Volume Analyzed: 200, 250, 350, 400 ml.

Enrichment Broths, Temperature, Incubation: T = Tetrathionate, 41.5°C, 20 ± 2 h; S = Selenite, 41.5°C, 20 ± 2 h.

Plating Agars, Temperature, Incubation: B = Brilliant Green Agar, 41.5°C, 20 ± 2 h; X = XLD Agar, 41.5°C, 20 ± 2 h.

** NEG: Negative.

MONITORING OF STORM WATER RUNOFFS FOR BACTERIAL AND VIRAL PATHOGENS OF MAN

by

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Microbiology Laboratories
Applied Research Division
Canada Centre for Inland Waters
Environment Canada

Project No. 76-8-41

Investigation of Pathogens
for
Urban Drainage Subcommittee
Canada-Ontario Agreement on
Great Lakes Water Quality

ABSTRACT

An eight-month project (April - November 1976) was undertaken to study the distribution of bacterial pathogens and viruses in storm water runoffs at the Malvern storm sewer outfall in central Burlington. All samples were hand collected over a four-hour period, with zero-hour sample collection occurring when a change in the normal infiltration flow was noticed. The samples were analyzed for total coliforms, fecal coliforms, fecal streptococci, heterotrophs, fungi, Pseudomonas aeruginosa, Leptospira, salmonellae and viruses. Salmonellae were isolated over the four-hour study period and one sample collected from the latter part of an event was positive for virus (reovirus). No human pathogen Leptospira were isolated.

Data from this study indicate that it is not possible to predict the time when peak microbial populations occur in runoff waters or the appearance of human pathogens.

RÉSUMÉ

On a entrepris d'étudier pendant huit mois (d'avril à novembre 1976) la répartition des bactéries pathogènes et des virus dans les eaux de ruissellement de l'émissaire pluvial de Malvern, dans le centre de Burlington. Tous les échantillons ont été prélevés à la main, en quatre heures, à partir du moment où l'on a noté une modification du débit d'infiltration. L'analyse des échantillons a porté sur les coliformes ainsi que sur les coliformes et les streptocoques d'origine fécale, sur les bactéries hétérotrophes, les champignons, la Pseudomonas aeruginosa, la Leptospira, les salmonelles et les virus. Des salmonelles ont été isolées tout au cours des quatre heures et l'un des échantillons prélevés à la fin de cette période a révélé la présence de réovirus. Aucune Leptospira pathogène pour l'homme n'a été décelée.

D'après les données de cette étude, il paraît impossible de prévoir le moment où la population microbienne sera à son maximum dans les eaux de ruissellement ni celui où des microbes humains seront présents.

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CONCLUSIONS

1) Manually collected samples of storm runoffs tend to produce lower maximum bacterial densities and delayed peaking of populations due to (a) immediate refrigeration of samples after collection inhibiting further growth, and (b) sample collection at time of first flow change compared to 5 cm rise in water level required to trigger automatic samplers.

2) Infiltration samples continue to be a source of low grade continuous pollution.

3) There is no predictable pattern for maximum bacterial population occurrence.

4) Pathogens were isolated during all phases of the 240-minute studies. Thus, no predictable pattern of pathogen appearance could be established.

5) The unpredictable distribution pattern of peak microbial populations and pathogen incidence do not encourage the concept of collecting and treating a specific portion of each storm runoff to decrease the potential health hazards in recipient waters.

6) Studies should be carried out to establish the extent of bacterial loading in inshore receiving waters, the extent of this loading in the main water body, and the duration of each storm's effect.

1. INTRODUCTION

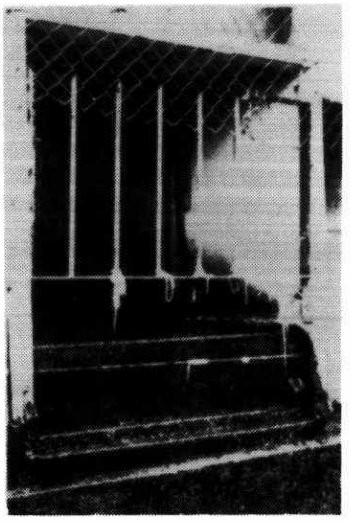
The presence of high densities of pollution indicator bacteria in Ontario storm water runoffs was confirmed in a previous study by Dutka and Rybakowski [1]. Data from this study support literature [2,3] indicating that bacterial densities in storm water runoffs are similar to dilute sewage and, therefore, constitute a health hazard.

Studies on the sources of bacteria found in runoffs from residential and light commercial areas indicated that the bacteria were predominantly of non-human origin [3,4]. However, in several instances [1,3,5] bacterial pathogens were isolated in runoff waters, demonstrating the existence of potential health hazards. Whether this hazard exists throughout the life of a rainfall, or only occurs during a specific portion of the rainfall, has not been sufficiently documented.

If pathogenic organisms only occur during a specific storm period, it might be feasible to collect and treat these waters and allow other non-pathogen containing waters to reach receiving waters unobstructed and untreated. However, before any decision can be made on this hypothesis, data must be obtained on the time-distribution of pathogens in storm runoff waters. Therefore, a study, using hand-collected samples, was undertaken to try and establish the distribution pattern of bacterial and viral pathogens in storm runoff waters.

1.1 Study Site

A storm sewer outfall on Malvern Road in Burlington, Ontario was selected as the study site. This outfall empties directly into a small creek (Figure 1) and was the site of a previous study [1]. The Malvern Road area is a 23.3 ha section in central Burlington. The entire area is zoned as single-family residential and there are no vacant lots within the area. Most of the houses were build in the early 1960's with virtually all development completed by 1964. The majority of the houses in this area have their eavestroughs connected directly to storm sewers which are 100% separated.



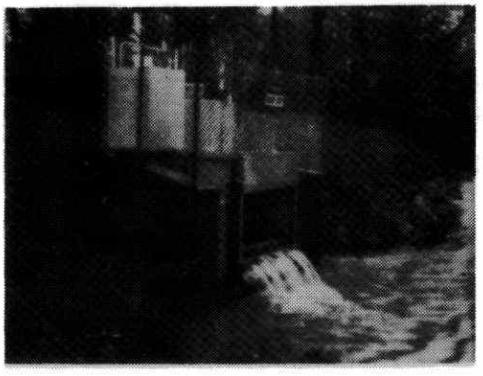


FIGURE 1. MALVERN STORM SEWER SAMPLING SITE, BURLINGTON, ONTARIO. AREA DRAINED IS FULLY DEVELOPED WITH SINGLE-FAMILY DWELLINGS

2 MICROBIOLOGICAL SAMPLE COLLECTION PROCEDURES

2.1 Storm Water Samples

Routine microbiological samples for coliforms, fecal coliforms, fecal streptococci, P. aeruginosa, fungi, heterotrophs, salmonella/100 ml and Leptospira/100 ml were hand-collected at the weir outfall and in the creek, five metres downstream of the outfall. At the first indication of rainfall, a technician would arrive at the sampling site and wait for the first drops of rain. When the rain started, the first samples would be collected as soon as there was a noticeable change in the flow rate and this sample would be labelled 0-hour. Weir samples were collected at 0, 10, 20, 30, 45, 60, 75, 90, 105, 150, 180 and 240 minutes. Creek samples were collected at 0, 30 and 120 minutes. Samples were collected in sterile glass 500-ml bottles and iced immediately after collection. All samples were processed within 18 hours.

2.2 Infiltration Samples

Ten hand-collected samples of infiltration waters were collected at the weir mouth over a seven-month period (May 10-November 30). After collection, samples were iced, returned to the laboratory, and tested within four hours of collection.

2.3 Salmonella Collection Pad Samples

Salmonella collection pads [6] were suspended in the storm water for the following time periods during each event: 0-30, 30-60, 60-120, and 120-240 minutes. After removal from the storm water, the five fingers from each pad were cut free from their frame, placed in sterile "Whirl-Pak" bags, iced and returned to the laboratory for processing within 18 hours of collection.

2.4 Virus Collection Pad Samples

Virus collection pads, which were similar to the salmonella collection pads, were suspended in the storm water during five events for the following time periods: 0-30, 30-60, 60-120 and 120-240 minutes. After removal from the storm water, the fingers were cut free from their frame, placed in sterile "Whirl-Pak" bags, packed in ice and shipped within 18 hours to Ottawa for processing.

3 MICROBIOLOGICAL METHODS

Total coliform, fecal coliform, fecal streptococcus, Pseudomonas aeruginosa and fungus density estimates were performed on all samples, using the membrane filtration technique (MF) and media described in "Methods for Microbiological Analysis of Waters, Wastewaters and Sediments" [6]. Heterotroph densities were also performed on all samples using the spread plate technique [6].

Semi-quantitative salmonella estimations were performed on all storm water and infiltration samples. One hundred-ml aliquots of storm water were filtered and the filters placed in 100-ml aliquots of tetrathionate and selenite broths prewarmed to 41.5°C and into nutrient broth prewarmed to 35°C. After 24 hours of incubation at 35°C, 10-ml aliquots of nutrient broth were placed directly into 100-ml aliquots of tetrathionate and selenite broths for 24 hours at 41.5°C. Cultivation and isolation procedures as outlined in "Methods for Microbiological Analysis of Waters, Wastewaters and Sediments" [6] were then followed. Salmonella collection pads were treated as outlined in the above manual.

3.1 Virus Methods

Processing of the gauze collection pads was initiated within 24 hours of collection. The pads were extracted while still in the bags by expressing the water from the pads, raising the pH to 8.0 and adding sufficient fetal calf serum to give a 10% serum concentration. The expressed alkaline-serum treated water was poured back into the bags, and mixed with the gauze pad. The mixture was expressed from the pad, collected, neutralized to pH 7.2 and centrifuged. The supernatant collected was concentrated by hydroextraction with polyethylene glycol. The concentrate was inoculated into monolayers of BS-C-1 and BGM cells as outlined by Sattar and Westwood [7] and examined for the presence of viruses.

3.2 Leptospira Methods

Water samples for isolation of pathogenic Leptospira were tested by Standard Biological Laboratories Limited, Mississauga, Ontario by the following procedure: each of the 40 water samples was treated with 5-fluorouraul to eliminate contaminating bacteria and then samples

were applied to the surface of E.M. semisolid medium [8]. The resulting growth in Dingers zone was examined microscopically for Leptospira species from 72 hours onward. Serological tests were performed on positive cultures to test for pathogenic Leptospira.

Samples of water were inoculated into hamsters and guinea pigs and the animals were observed over 14 days, then sacrificed to check for any evidence of icterus or pyrexia. When findings were negative, blood was collected from all animals and the sera tested against all known pathogens by agglutination-lysis tests.

3.3 Isolates

Twenty-five coliform and 25 fecal coliform isolates were collected from five infiltration samples during the July-November period to typify the normal enteric population of infiltration waters. Three sets of 25 coliform and 25 fecal coliform isolates were also collected during five different storms throughout the following collection periods: 0-30, 60-90 and 120-240 minutes, for a total of 75 coliform and 75 fecal coliform isolates per storm. Isolate identification procedures were performed as outlined by Dutka and Tobin [9].

4 RESULTS

A summary of all runoff data is presented in Table A1 (a-f) and Figures A1-A11 of the Appendix. Figure 2 presents the mean data of 12 events; Table 1 presents mean data collected from the eight 240-minute storms, and Figure 3 illustrates an event (Storm #9) which was very similar to the mean storm (Figure 2).

During the 1975 study [1] of this storm sewer outfall it was noted that microbial populations reached their maximum levels within the first 45 minutes of an event. In this study (Figures 2 and 3), maximum bacterial populations tended to occur 60 to 105 minutes after the water flow in the weir was noticed to change. This event occurred usually within 5-15 minutes of a rainfall commencing, depending on the heaviness of the initial fall. Part of the difference noted between 1975 and 1976 observations of peak population occurrence may be due to sample collection techniques, in that 1975 samples were collected by an automatic sampler which was triggered to start sampling by a 5 cm rise in water level. A light rain could take up to one hour to trigger the sampling mechanism because of the slow build up within the storm sewer.

Another difference noted between the 1975 and 1976 studies was the general trend to lower bacterial densities during each event in the 1976 study. This is believed, again, to be related to sample collection technique. When the samples were collected automatically, there was often a 10 to 15 hour delay before the samples were retrieved and iced, especially during late evening rains. This delay in collection and icing the samples may have been sufficient to allow some multiplication to take place within the collection bottles. The microbial populations with the greatest decrease from 1975 levels were Pseudomonas aeruginosa and fungi, both of which respond fairly rapidly to nutrient enrichment.

Traditional indicator densities, such as coliforms, fecal coliforms and fecal streptococci approached dilute sewage concentrations during each event, thus re-emphasizing the health hazards due to pathogenic bacteria and viruses which are contained within these storm runoff waters.

TABLE 1. MICROBIOLOGICAL MEAN DATA FROM MALVERN ROAD STORM SEWER, BURLINGTON, JUNE 20 - OCTOBER 24, 1976.

Dates of Storms 1976	Time of Sample Collection (min.)	Coliforms /100 ml	Fecal Coliforms /100 ml	Fecal Streptococci /100 ml	*P. aeruginosa* /100 ml	Fungi /100 ml	Heterotroph Count /ml
June 20	0	2.3×10^3	6.7×10^2	1.4×10^3	36	6.0×102^2	7.5×10^4
June 24	10	2.8×10^3	5.2×10^2	3.3×10^3	38	1.8×10^3	9.3×10^4
Aug. 5	20	2.5×10^3	5.9×10^2	5.5×10^3	36	7.8×10^2	6.9×10^4
Aug. 13	30	2.4×10^3	8.9×10^2	3.3×10^3	88	5.1×10^3	8.0×10^4
Sept. 21	45	2.5×10^3	5.7×10^2	1.2×10^3	86	1.4×10^4	1.5×10^5
Oct. 6	60	1.1×10^4	5.2×10^3	7.6×10^3	250	3.0×10^4	4.3×10^5
Oct. 19	75	8.1×10^3	3.3×10^3	5.5×10^3	270	3.5×10^4	4.9×10^5
Oct. 24	90	7.9×10^3	2.9×10^3	6.5×10^3	410	3.2×10^4	2.8×10^5
	105	1.8×10^4	3.2×10^3	6.5×10^3	250	2.6×10^4	3.4×10^5
	120	1.5×10^4	3.1×10^3	4.4×10^3	330	1.9×10^4	2.7×10^5
	150	1.1×10^4	3.4×10^3	4.9×10^3	290	1.7×10^4	3.5×10^5
	180	9.6×10^3	5.7×10^3	5.7×10^3	260	1.6×10^4	3.1×10^5
	240	7.5×10^3	4.3×10^3	4.2×10^3	240	1.9×10^4	1.7×10^5

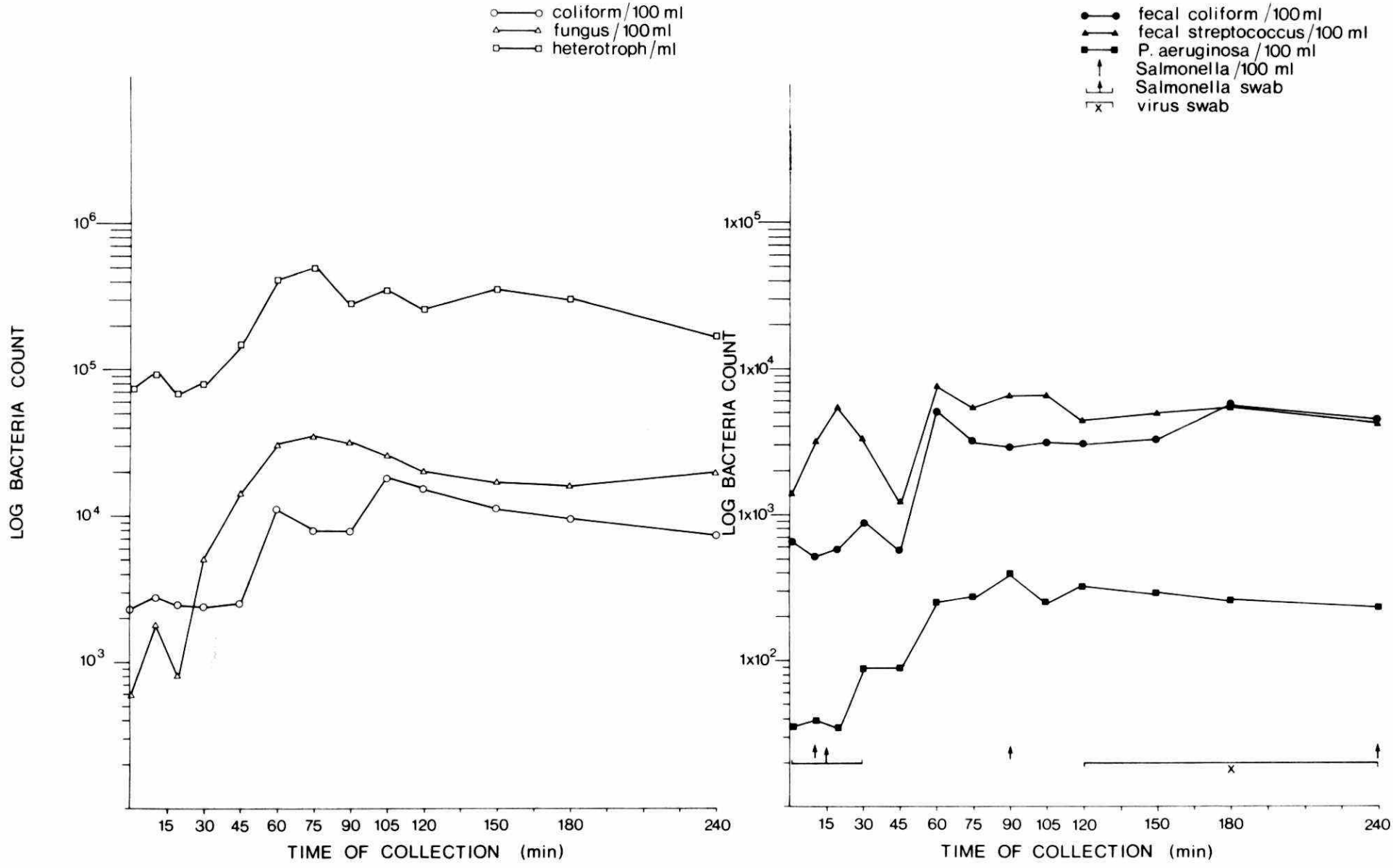


FIGURE 2. MALVERN STORM SEWER STUDY MEAN DATA, 12 EVENTS, APRIL 21 - OCTOBER 24, 1976

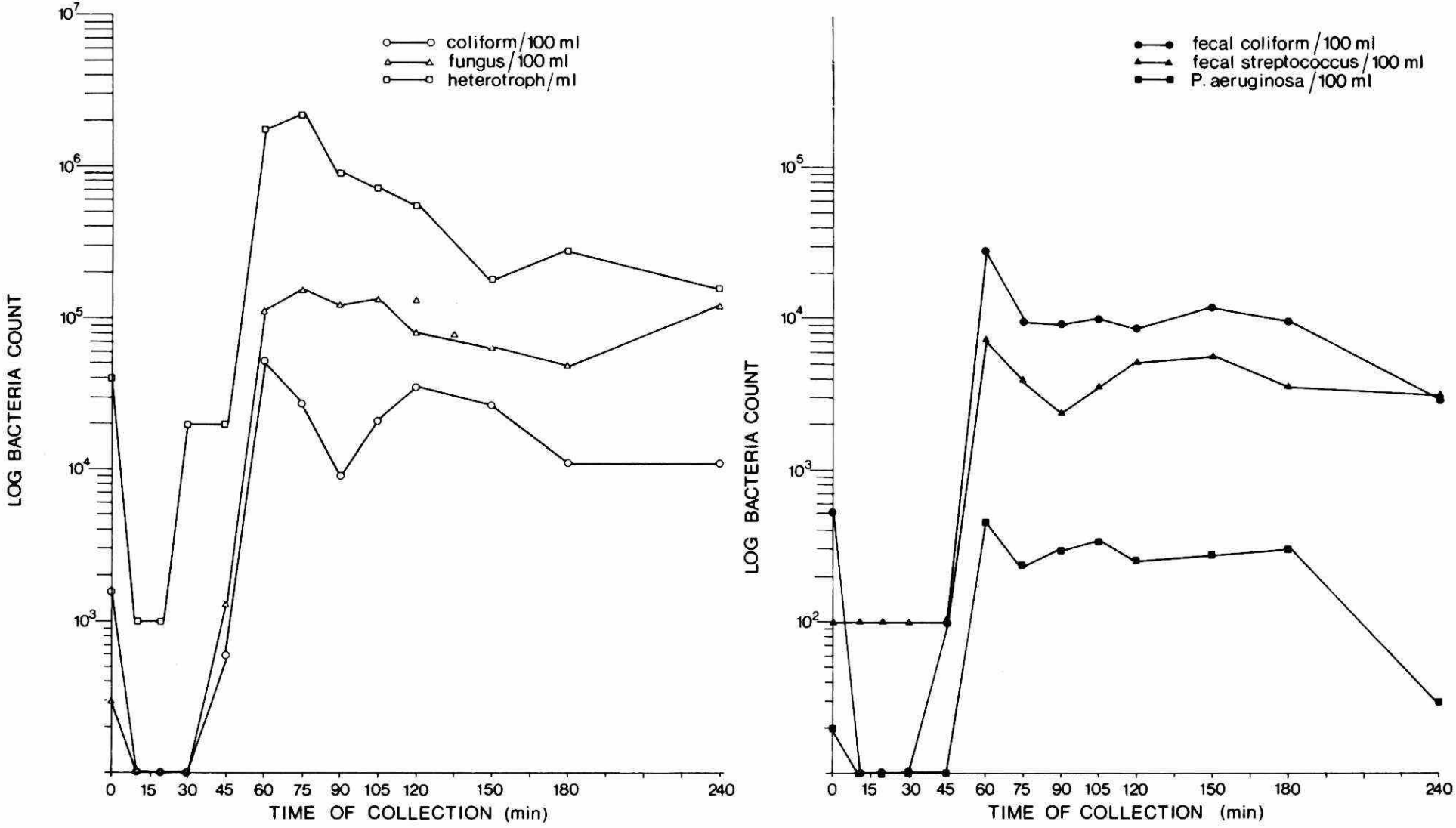


FIGURE 3. MALVERN STORM SEWER STUDY RAINFALL #9 OCTOBER 6, 1976

One of the indicator organisms used in this study, P. aeruginosa, is also a pathogen. This organism has been implicated in eye, ear, skin and upper respiratory tract infections in bathers. Thus, the increased P. aeruginosa populations discharged into receiving waters, many of which are used for recreational purposes, constitute an elevated risk not previously suspected.

The mycological procedures used during this study did not differentiate between invasive pathogenic fungi and saprophytic fungi. It is known that both types will grow on the media used. Therefore, the striking increase seen in fungal populations during most rainfalls should be cause for concern especially as many of the invasive fungi do so through skin contact. Thus, storm runoff waters are contributing another previously unsuspected group of potential pathogens into receiving and recreational waters.

Heterotrophic bacterial populations were studied as indicators of total bacterial input. During most storms it can be seen that this parameter generally followed the patterns of the other indicator bacteria.

From Figures A1-A11 (Appendix), it can be seen that there are no typical rainfalls. Each event is unique, with its own unpredictable flow pattern. Event #3 (Figure 3) produced the latest storm peaks while events, #5 and #10 (Figures 5 and 9) produced the earliest maximum populations.

The haphazard distribution pattern of microbial populations in storm runoff waters, combined with the variability of pathogen appearance (Figure 2) tends to discourage the concept of collecting that portion of storm runoff waters which contains the maximum bacterial load and pathogens and holding these waters for treatment. To adopt this philosophy, it would be necessary to collect for treatment the waters from the first 2.5 to 3 hours of each event. This is logistically and economically impractical.

4.1 Pathogen Isolations

Only non-pathogenic Leptospira were found in all samples tested. It is clear that the sampled waters are able to support pathogenic Leptospira. However, pathogenic Leptospira are much more likely to be

encountered during periods of drought and in stagnant pools where adequate concentrations for enumeration can build up. If human pathogen Leptospira were to be found, the 0-hour samples would be the most likely site. It is believed that pathogenic Leptospira are more delicate than the non-pathogenic Leptospira and that the turbulence and movement of water in flood periods might tend to interfere with their survival.

Salmonellae were isolated on four occasions from 100-ml grab samples collected at the 10, 90 and 240-minute marks. Isolations of salmonella were also made from the 0-30 minute collecting pads on two occasions, May 30 and July 7, 1976.

The detection of S. thompson during the May 30 and July 7 storms is indicative of a local carrier, either domestic pet or wild animal. The finding of S. typhimurium at the 90 and 240-minute marks of the October 28 event and the 0-hour mark of the October 6 storm sample would indicate that this species was endemic in the sampling area, and that there are probably several contributory sources.

Salmonellae were found twice during this study within the first 30 minutes of a storm starting, twice at the 90-minute mark, and once at the 240-minute mark. It appears that hazardous organisms are discharged throughout the life of an event and probably from several sources.

Some microbiologists have found that a pre-enrichment procedure (nutrient broth) enhances the recovery of salmonellae from environmental samples. In this study, no instances were found where pre-enrichment cultures were positive, while the direct enrichment (tetrathionate and selenite broths) procedure proved to be negative. Contrarily, only in one instance was a positive result obtained from a pre-enriched sample and the sample was also positive in one of the routinely used enrichment broths. In 188 attempts, there were six positive results by conventional methods and one positive (one of the six) by pre-enrichment methods. No pre-enrichment procedures appeared to be required.

An enteric virus was isolated only once during the study period, on May 30. This virus, a reovirus, was isolated on a collecting pad suspended in the weir during the last two hours (120-240 minutes) of a minor event (9 mm rainfall), when the water flow had almost returned to normal.

Reoviruses are enteric viruses which are very similar to enteroviruses with two important exceptions. They are much larger in size and contain a double-stranded RNA core. Reoviruses have been isolated from surface waters and are known to be causally involved in human infections.

Again, the finding of this human pathogen during the latter part of a storm strongly supports the conclusion that hazardous organisms are discharged non-predictably, throughout the life of a rainfall and subsequent runoff period. Therefore, the proposal that only pathogen-containing runoff waters be contained and treated would not be feasible, based on the results of this study. If treatment of runoff waters were undertaken, all runoff waters would have to be treated.

4.2 PA:FC Ratios

Recent studies by Cabelli, Kennedy and Levin [10] on Pseudomonas aeruginosa and fecal coliform densities in estuarine and fresh recreational waters indicated that the ratios between these organisms might be used to typify their probable source. Their results tended to show that PA:FC ratios (x 100) of more than 20 were found when fecal wastes were not the source of the fecal coliforms. Also when PA:FC ratios (x 100) of approximately 0.1 were found, coupled with fecal coliform densities of more than 1000, the probable source was animal feces. Based on this hypothesis and examination of Table A1 (Appendix) and Table 2, the April 21 and July 7 storm water bacterial populations were probably the result of land wash, as was suspected, and not direct fecal wastes. However, all other storm water events indicate a variety of pollution sources, i.e., human and animal feces, and landwash, within each event.

Because of the nature of the tested storm sewer and its catchment area, it is highly unlikely that human fecal material is involved in or is affecting the PA:FC ratios. The main contributors to PA:FC ratio would be animal and bird feces, and soil runoff. It is known (visual sighting) that racoons and muskrats make temporary homes in this storm sewer. Therefore, the mixed ratios detected are probably an accurate indication that the fecal coliform and P. aeruginosa populations come from a variety of sources. It is suspected, however, that storm

TABLE 2. RATIO OF *P. AERUGINOSA* TO FECAL COLIFORMS (PA:FC x 100) THROUGHOUT SAMPLE COLLECTION PERIOD, MALVERN STORM SEWER, 1976.

Date	Time of Collection in Minutes												
	0	10	20	30	45	60	75	90	105	120	150	180	240
April 21	≃ 20.0	≃ 25.0	≃100.0	666.7	359.0	159.1	218.8	142.1	333.3	263.2	122.7	283.3	
Creek				130.0						7.0			
May 30	≃100.0	≃ 11.1	3.7	7.1	2.0	4.4	5.0	3.0	1.6	4.0	5.0		
Creek				2.5						0.2			
June 20	22.0	57.1	21.7	14.3	53.7	4.6	8.1	7.2	5.6	6.3	13.6	2.1	0.8
Creek				0.6						3.1			
June 24	3.3	10.0	20.0	6.7	6.7	30.0	1.7	35.0	33.3	45.0	16.0	≃800.0	20.0
Creek	0.8			2.9						2.2			
July 7	50.0	148.0	13.3	62.5	230.8	192.9	291.7	194.4	388.8	213.3	328.6		
Creek	4.5			83.0						1.5			
Aug. 5	6.5	11.8	16.3	64.3	25.0	36.4	87.5	102.4	91.5	264.7	90.9	33.3	254.5
Creek	1.8			13.1						6.7			
Aug. 13	7.7	≃ 16.6	11.1	14.3	≃ 20.0	90.0	19.1	111.1	62.5	43.8	53.3	50.0	92.3
Creek	27.3			80.0						97.6			
Sept. 21	5.0	60.0	62.5	220.0	442.9	42.3	12.1	7.8	13.5	12.1	21.7	23.0	15.9
Creek	5.3			115.0						21.0			
Oct. 6	3.8	≃100.0	≃100.0	≃100.0	10.0	1.6	2.5	3.2	3.4	3.0	2.3	3.2	1.0
Creek	≃ 2.0			300.0						0.7			
Oct. 13	≃ 5.8	1.3	0.4	8.8	1.1	0.9	0.9						
Creek	≃ 16.7			5.8			0.6						
Oct. 19	≃ 5.0	≃ 5.0	≃ 10.0	≃ 10.0	≃ 10.0	3.3	6.7	1.9	0.9	1.9	2.0	0.6	2.1
Creek	≃ 1.3			1.7						0.6			
Oct. 24	≃ 0.6	8.0	0.3	0.4	0.9	1.0	1.1	0.5	0.9	39.5	34.4	18.2	22.2
Creek	≃ 1.4			≃ 0.3						1.3			

waters present a set of unstable short-term conditions to which the ratio concept of Cabelli, et al [10] is not fully applicable.

4.4 Creek Samples

Figure 4 and Table 3, and Figure A12 (Appendix) present the microbiological data obtained from the creek samples during most of the events studied. From these data, especially Table 3 and Figure A12 (Appendix), it can be seen that each rainfall had a variety of effects on the stream's microbial population. Sometimes maximum populations occurred at the 30-minute mark as shown in the October 24 event. More often the maximum bacterial populations were found at the 120-minute mark.

In most instances there were no significant differences in creek microbiological populations during the 120-minute sampling period, even though numerical examination of the data indicates a population elevation of most parameters by the 120-minute mark. This may be due to the following:

1) dilution by both rainfall and storm runoff waters,
2) the location of the sampling site in relation to the storm sewer,
3) association of bacteria with particles in the runoff which may sink, settle or tumble along the stream bottom, shedding bacteria haphazardly,
4) variations in the numbers of bacteria which are particle-associated (each particle would show only one or two obvious colonies because of the filtration procedure used, although the particle-associated bacteria may number from one to 100).

Therefore, the observed creek data may not reflect actual conditions throughout the length of the creek.

The isolation of S. infantis at the 120-minute mark of the October 13 event was not unexpected. This indicates that the salmonella concentration was at least one organism per 100 ml. Furthermore, the population was not homogeneously dispersed throughout the creek and runoff water; the organisms only grew from one of the three 100 ml aliquots tested. The finding of S. typhimurium at 0-time during the

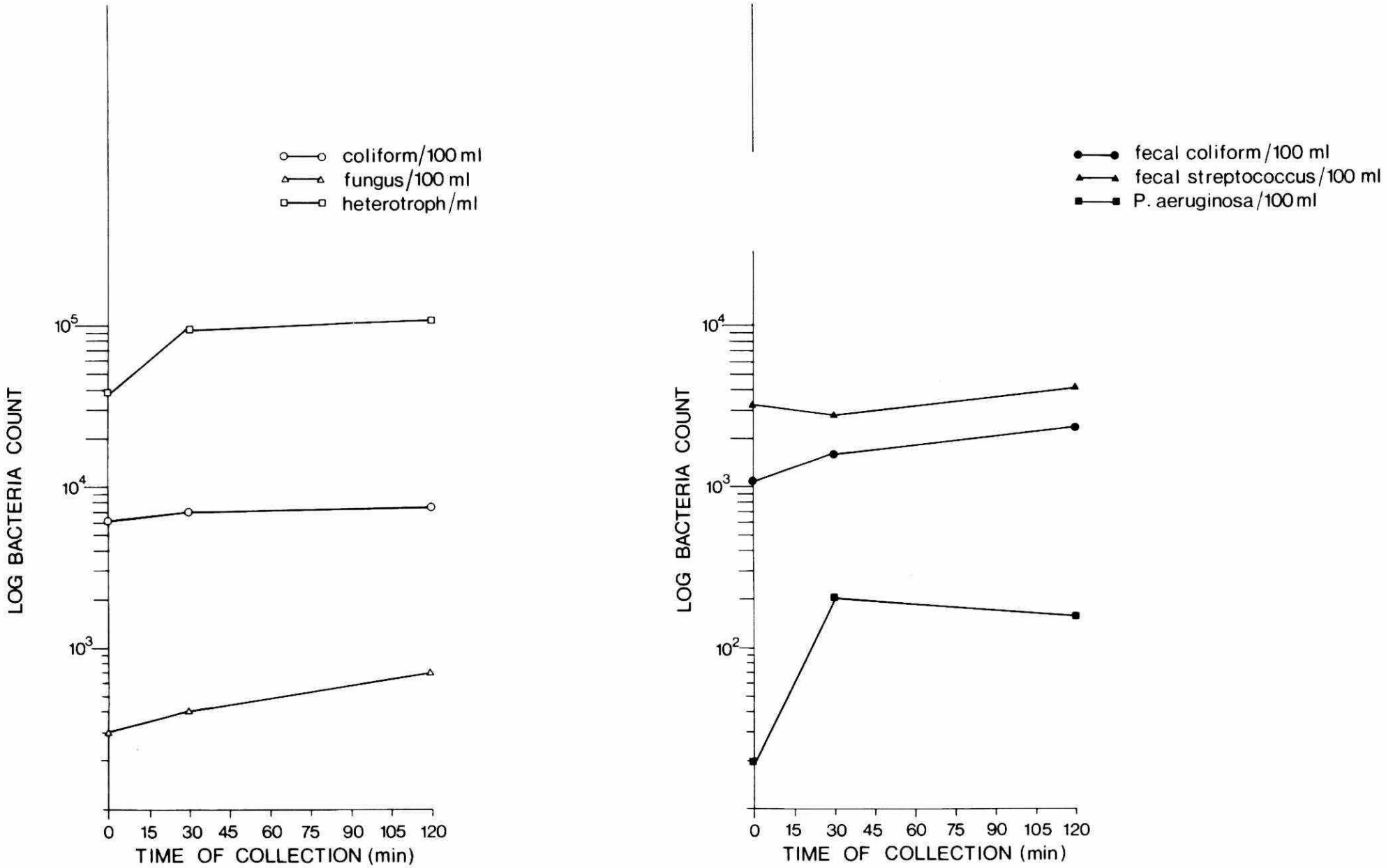


FIGURE 4. EFFECT OF MALVERN STORM SEWER RUNOFF ON DOWNSTREAM CREEK, TYPICAL RAINSTORM (AUGUST 5, 1976)

TABLE 3. MICROBIOLOGICAL DATA FROM MALVERN ROAD CREEK, FIVE METRES DOWNSTREAM OF OUTFALL, APRIL 21 - OCTOBER 24, 1976

Storm Date 1976	Creek Sample	Time of Sample Collection	Coliforms /100 ml	Fecal Coliforms /100 ml	Fecal Strepto-cocci /100 ml	P. aeruginosa /100 ml	Fungi /100 ml	Heterotroph Count /ml	Salmonella /100 ml
April	30 min	13:15	2.3×10^3	3.9×10^2	1.9×10^3	5.1×10^2	1.6×10^3	1.4×10^5	Neg.
21	120 min	14:45	2.4×10^3	7.1×10^2	8.7×10^2	50	5.0×10^2	1.2×10^5	Neg.
May	30 min	14:25	1.5×10^3	4.0×10^2	1.3×10^3	10	1.2×10^4	5.4×10^6	Neg.
30	120 min	15:55	5.3×10^4	5.2×10^3	1.1×10^4	< 10	9.0×10^3	4.2×10^6	Neg.
June	30 min	19:10	4.0×10^3	1.6×10^3	1.4×10^3	10	1.9×10^4	5.3×10^4	Neg.
20	120 min	20:40	2.5×10^3	1.6×10^3	2.4×10^3	50	4.4×10^3	7.0×10^5	Neg.
June	0 min	10:30	4.4×10^3	1.3×10^3	3.5×10^3	10	7.0×10^3	3.5×10^5	Neg.
24	30 min	11:00	2.4×10^3	7.0×10^2	1.9×10^3	20	9.0×10^3	2.1×10^5	Neg.
	120 min	12:30	4.9×10^3	2.7×10^3	3.3×10^3	60	2.9×10^3	3.5×10^5	Neg.
July	0 min	18:15	1.8×10^3	4.4×10^2	3.8×10^2	20	2.6×10^2	5.3×10^4	Neg.
7	30 min	18:45	1.6×10^4	1.8×10^3	1.4×10^4	1.5×10^3	3.2×10^5	3.8×10^5	Neg.
	120 min	20:15	2.4×10^4	1.3×10^4	5.5×10^3	2.0×10^2	4.3×10^4	7.4×10^5	Neg.
Aug.	0 min	16:25	6.3×10^3	1.1×10^3	3.2×10^3	20	3.0×10^2	3.8×10^4	Neg.
5	30 min	16:55	7.0×10^3	1.6×10^3	2.8×10^3	2.1×10^2	4.0×10^2	9.6×10^4	Neg.
	120 min	18:25	7.5×10^3	2.4×10^3	4.3×10^3	1.6×10^2	7.0×10^2	1.1×10^5	Neg.

TABLE 3. (CONT'D)

Storm Date 1976	Creek Sample	Time of Sample Collection	Coliforms /100 ml	Fecal Coliforms /100 ml	Fecal Strepto-cocci /100 ml	P. aeruginosa /100 ml	Fungi /100 ml	Heterotroph Count /ml	Salmonella /100 ml
Aug.	0 min	07:40	2.6×10^3	1.1×10^3	1.5×10^3	3.0×10^2	7.0×10^2	3.2×10^4	Neg.
13	30 min	08:10	1.9×10^3	2.5×10^2	2.3×10^3	2.0×10^2	8.0×10^2	2.6×10^4	Neg.
	120 min	09:40	4.2×10^3	8.2×10^2	2.1×10^3	8.0×10^2	4.0×10^3	8.4×10^4	Neg.
Sept.	0 min	18:15	7.1×10^3	5.7×10^2	1.2×10^3	30	1.3×10^3	2.6×10^5	Neg.
21	30 min	18:45	3.6×10^3	2.0×10^2	1.1×10^3	230	2.3×10^3	1.0×10^5	Neg.
	120 min	20:15	1.4×10^4	1.0×10^3	2.5×10^3	210	2.1×10^3	1.4×10^5	Neg.
Oct. 6	0 min	15:00	5.3×10^3	5.1×10^2	<100	< 10	5.5×10^3	1.6×10^5	S. typhi murium
	30 min	15:30	1.0×10^2	60	<100	1.8×10^2	1.5×10^4	3.6×10^4	Neg.
	120 min	17:00	6.1×10^4	2.2×10^4	3.4×10^3	1.6×10^2	5.6×10^4	2.0×10^5	Neg.
Oct.	0 min	15:50	1.3×10^3	60	< 10	< 10	8.0×10^2	1.5×10^5	Neg.
13	30 min	16:20	9.0×10^4	1.9×10^3	7.0×10^2	1.1×10^2	1.8×10^4	1.2×10^5	Neg.
	120 min	17:05	3.5×10^4	1.6×10^3	2.6×10^3	10	1.0×10^4	1.8×10^5	S. infantis
Oct.	0 min	16:40	4.2×10^3	8.0×10^2	1.4×10^2	< 10	4.0×10^2	6.0×10^4	Neg.
19	30 min	17:10	3.3×10^3	6.0×10^2	1.0×10^2	10	6.0×10^2	6.8×10^4	Neg.
	120 min	18:40	3.0×10^4	3.5×10^3	2.7×10^3	20	6.0×10^3	2.4×10^5	Neg.
Oct.	0 min	08:50	3.0×10^3	7.0×10^2	1.7×10^3	< 10	3.0×10^2	8.0×10^4	Neg.
24	30 min	09:20	6.4×10^3	3.0×10^3	3.2×10^3	< 10	9.0×10^2	1.0×10^5	Neg.
	120 min	10:50	3.0×10^3	8.0×10^2	1.8×10^3	10	1.2×10^3	8.0×10^4	Neg.

October 6 event indicates that animals are a very important source of pathogens. Thus, even though man's pollution contributions are contained, animals and birds are very important in the maintenance of pathogens and the establishment of a potential health hazard to man in these waters.

4.5 Infiltration Samples

Table 4 presents the results of the analysis of ten infiltration samples collected between May 10 and November 30, 1976. Bacterial densities encountered during the 1976 study were very similar to those found in 1975 [1], with the exception that in 1976 maximum populations occurred five to six weeks after the 1975 July maximums. Thus, the infiltration waters continued to be a source of light but consistent pollution into the recipient creek.

4.6 Isolates

Table 5 presents a summary of infiltration and storm sewer isolate data found in Tables A2 and A3 of the Appendix. In this study of five infiltration samples, higher E. coli levels and lower Citrobacter populations were found compared to the single 1975 study. However, the isolate distribution pattern was similar to the 1975 study and is typical of waters subjected to fecal pollution with some regrowth within the system as typified by the fairly high Klebsiella population.

Storm runoff isolates presented a more mixed population with higher Citrobacter and Enterobacter populations, typical of land wash. E. coli populations, although fluctuating from storm to storm and within storm periods (0-30, 60-90 and 120-240 minutes) (Table A2, Appendix), showed a consistent distribution pattern over the duration of the storms (Table 5). This was also found for the other three enterobacteriaceae.

Examination of each storm as an individual event indicated that maximum microbial populations tended to occur during the 60-105 minute period of most storms, with no typical enterobacteriaceae distribution pattern emerging during this time period. The only distribution patterns which appear are the increase in E. coli populations during the late summer and fall and the decrease in Klebsiella populations during this same time period.

TABLE 4. INFILTRATION SAMPLES, MALVERN ROAD STORM SEWER, BURLINGTON, 1976.

Date	Coliforms /100 ml	Fecal Coliforms /100 ml	Fecal Strepto-cocci /100 ml	P. aeru-ginosa /100 ml	Fungi /100 ml	Heterotroph Count /ml	Salmonella /100 ml	P. aeruginosa: Fecal Coliform Ratio x 100
May 10	3.0	< 1	13.0	< 1	13.0	4.0×10^3	Neg.	≈100.0
May 30	9.0	3.0	16.0	3.0	9.0	1.2×10^5	Neg.	100.0
June 14	5.8×10^2	7.0	3.4×10^2	3.9×10^2	80.0	1.7×10^5	Neg.	5570.0
July 20	4.4×10^2	1.2×10^2	1.4×10^2	10	60.0	3.3×10^4	Neg.	8.0
Aug. 17	5.3×10^3	1.3×10^2	9.0×10^2	7.0×10^2	1.0×10^2	6.6×10^4	Neg.	538.0
Aug. 30	4.0×10^3	1.4×10^3	3.4×10^2	7.4×10^2	43.0	8.1×10^3	Neg.	53.0
Sept.29	5.6×10^3	1.8×10^3	1.2×10^2	10.0	1.2×10^2	9.0×10^3	Neg.	.6
Oct. 12	6.0×10^2	2.0×10^2	40.0	9.0	4.0×10^2	1.6×10^4	Neg.	4.5
Nov. 2	4.5×10^2	83.0	1.1×10^2	< 1	80.0	1.1×10^4	Neg.	≈100.0
Nov. 30	1.2×10^2	11.0	21.0	33.0	13.0	5.9×10^3	Neg.	300.0

TABLE 5. SUMMARY OF COLIFORM AND FECAL COLIFORM BIOTYPES ISOLATED FROM MALVERN ROAD STORM SEWER INFILTRATION SAMPLES, 1976.

Species or Genera	Coliform			Fecal Coliform		
	No.	%	% EC+	No.	%	% EC+
E. Coli	74	60	78	125	100	94
Klebsiella	36	29	19			
Enterobacter	12	10	25			
Citrobacter	2	1	0			
Total	124	100		125	100	

SUMMARY OF COLIFORM AND FECAL COLIFORM BIOTYPES ISOLATED FROM MALVERN ROAD STORM SEWER, STORM SAMPLES,1976

Species or Genera	0-30 Minutes						60-90 Minutes						120-240 Minutes					
	Coliform			Fecal Coliform			Coliform			Fecal Coliform			Coliform			Fecal Coliform		
	No.	%	% EC+	No.	%	% EC+	No.	%	% EC+	No.	%	% EC+	No.	%	% EC+	No.	%	% EC+
E. coli	50	45	86	123	98	92	50	44	92	124	100	96	54	46	89	125	100	98
Klebsiella	26	23	15	2	2	50	36	31	6				34	29	6			
Enterobacter	25	22	16				18	16	17				22	19	9			
Citrobacter	11	10	0				10	9	0				8	6	0			
Total	112	100		125	100		100	100		124	100		118	100		125	100	

Fecal coliform data from both infiltration samples and storm runoff waters are surprisingly consistent in that almost 100% of all storm water runoff and 98-100% of infiltration sample isolates are E. coli, with 92-98% of the isolates giving a positive EC test (an indication of fecal origin). In contrast E. coli isolates collected from coliform plates varied from 78-96% EC positive.

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A P P E N D I X

TABLE A1 (a). MICROBIOLOGICAL DATA FROM MALVERN ROAD STORM SEWER, BURLINGTON, APRIL 21 - OCTOBER 24, 1976.

Date	Storm		Time of Sample Collection	Coliforms /100 ml	Fecal Coliforms /100 ml	Fecal Streptococci /100 ml	P. aeruginosa /100 ml	Fungi /100 ml	Heterotroph Count /ml	Salmonella /100 ml	Leptospira /100 ml	Time of Swab Collection	Salmonella Collecting Swab	Virus Collecting Swab
	Duration	Amount Rainfall												
April 21	180 min.	9 mm	12:45	1.6×10^{3}	50	2.8×10^{3}	< 10	2.0×10^{2}	2.0×10^{6}	Neg.		12:45-	Neg.	
			12:55	2.5×10^{3}	40	3.8×10^{3}	< 10	4.0×10^{2}	5.0×10^{5}	Neg.		13:15		
			13:05	1.2×10^{3}	10	2.8×10^{3}	< 10	9.0×10^{2}	2.2×10^{5}	Neg.				
			13:15	2.8×10^{3}	210	2.3×10^{3}	1.4×10^{3}	3.6×10^{3}	2.6×10^{5}	Neg.		13:15-	Neg.	
			13:30	2.0×10^{3}	390	4.3×10^{3}	1.4×10^{3}	1.1×10^{3}	1.2×10^{5}	Neg.		13:45		
			13:45	1.2×10^{3}	440	5.3×10^{3}	7.0×10^{2}	2.0×10^{3}	1.0×10^{5}	Neg.		13:45-	Neg.	
			14:00	1.6×10^{3}	320	4.8×10^{3}	7.0×10^{2}	2.0×10^{3}	8.4×10^{4}	Neg.		14:45		
			14:15	9.0×10^{2}	190	2.6×10^{3}	2.7×10^{2}	1.0×10^{3}	9.6×10^{4}	Neg.				
			14:30	1.2×10^{3}	180	1.6×10^{3}	6.0×10^{2}	1.5×10^{3}	5.2×10^{4}	Neg.				
			14:45	9.0×10^{2}	190	7.0×10^{2}	5.0×10^{2}	1.5×10^{2}	6.8×10^{4}	Neg.		14:45-	Neg.	
			15:15	1.3×10^{3}	220	3.0×10^{3}	2.7×10^{2}	1.3×10^{3}	1.0×10^{4}	Neg.		15:45		
			15:45	1.1×10^{3}	120	1.7×10^{3}	3.4×10^{2}	3.0×10^{2}	8.8×10^{4}	Neg.				
May 30	150 min.	9 mm	13:55	1.0×10^{2}	10	10	< 10	< 100	1.6×10^{5}	Neg.	Neg.	13:55-	S. thompson	Neg.
			14:05	2.0×10^{2}	90	5.0×10^{2}	< 10	6.0×10^{3}	2.0×10^{6}	Neg.	Neg.	14:25		
			14:15	9.0×10^{2}	8.0×10^{2}	1.5×10^{3}	30	9.0×10^{3}	4.8×10^{6}	Neg.				
			14:25	7.0×10^{2}	7.0×10^{2}	2.9×10^{3}	50	8.0×10^{3}	1.8×10^{7}	Neg.	Neg.	14:25-	Neg.	Neg.
			14:40	6.3×10^{3}	3.5×10^{3}	4.8×10^{3}	70	1.5×10^{4}	6.6×10^{6}	Neg.		14:55		
			14:55	2.6×10^{3}	1.8×10^{3}	5.9×10^{3}	80	1.6×10^{4}	2.0×10^{6}	Neg.	Neg.	14:55-	Neg.	Neg.
			15:10	9.0×10^{2}	1.4×10^{3}	7.9×10^{3}	70	9.0×10^{3}	1.8×10^{6}	Neg.		15:55		
			15:25	1.5×10^{3}	2.3×10^{3}	8.5×10^{3}	70	7.0×10^{3}	1.2×10^{6}	Neg.				
			15:40	1.5×10^{3}	6.0×10^{2}	3.6×10^{3}	10	8.0×10^{3}	2.0×10^{6}	Neg.				
			15:55	3.0×10^{3}	5.0×10^{2}	1.1×10^{3}	20	9.0×10^{3}	2.6×10^{6}	Neg.	Neg.	15:55-	Neg.	Reovirus
			16:25	1.0×10^{3}	2.0×10^{2}	4.0×10^{3}	10	5.0×10^{3}	2.2×10^{6}	Neg.		16:25		

TABLE A1 (b). MICROBIOLOGICAL DATA FROM MALVERN ROAD STORM SEWER, BURLINGTON, APRIL 21 - OCTOBER 24, 1976.

Date	Storm		Time of Sample Collection	Coliforms /100 ml	Fecal Coliforms /100 ml	Fecal Strepto-cocci /100 ml	P. aeru-ginosa /100 ml	Fungi /100 ml	Hetero-troph Count /ml	Salmonella /100 ml	Lepto-spira /100 ml	Time of Swab Collection	Salmonella Collecting Swab	Virus Collecting Swab
	Duration	Amount Rainfall												
June 20	240 min.	32 mm	18:40	$4.0x10^2$	$4.1x10^2$	$3.0x10^2$	90	$2.2x10^3$	$1.1x10^5$	Neg.	Neg.	18:40 - 19:10	Neg.	Neg.
			18:50	$5.1x10^2$	$1.4x10^2$	$4.0x10^2$	80	$6.0x10^2$	$8.3x10^4$	Neg.	Neg.			
			19:00	$3.5x10^2$	$2.3x10^2$	$1.0x10^2$	50	$2.4x10^3$	$6.6x10^4$	Neg.				
			19:10	$3.4x10^2$	$4.2x10^2$	$3.0x10^2$	60	$3.2x10^3$	$5.3x10^4$	Neg.	Neg.	19:10 - 19:40	Neg.	Neg.
			19:25	$2.0x10^3$	$4.1x10^2$	$1.3x10^3$	220	$6.5x10^4$	$2.3x10^5$	Neg.				
			19:40	$9.0x10^3$	$5.2x10^3$	$1.1x10^4$	240	$6.3x10^4$	$3.6x10^5$	Neg.	Neg.	19:40 - 20:40	Neg.	Neg.
			19:55	$6.1x10^3$	$2.7x10^3$	$5.5x10^3$	220	$7.4x10^4$	$6.0x10^5$	Neg.				
			20:10	$4.6x10^3$	$1.8x10^3$	$6.8x10^3$	130	$4.9x10^4$	$3.5x10^5$	Neg.				
			20:25	$2.7x10^3$	$2.5x10^3$	$1.0x10^4$	140	$3.6x10^4$	$8.6x10^5$	Neg.				
			20:40	$4.9x10^3$	$3.5x10^3$	$9.6x10^3$	220	$4.5x10^4$	$8.0x10^5$	Neg.	Neg.	20:40 - 22:10	Neg.	Neg.
			21:10	$5.4x10^3$	$4.2x10^3$	$1.2x10^4$	570	$5.2x10^4$	$3.9x10^5$	Neg.				
			21:40	$1.7x10^4$	$2.5x10^4$	$2.1x10^4$	530	$2.1x10^4$	$6.5x10^5$	Neg.				
			22:10	$6.9x10^3$	$2.4x10^4$	$2.0x10^4$	200	$2.5x10^4$	$4.0x10^5$	Neg.				
June 24	240 min.	101 mm	10:30	$5.0x10^2$	$3.0x10^2$	< 100	10	$1.0x10^3$	$1.9x10^4$	Neg.	Neg.	10:30 - 11:00	Neg.	
			10:40	$6.0x10^2$	$1.0x10^2$	100	10	$1.0x10^3$	$2.2x10^4$	Neg.	Neg.			
			10:50	$8.0x10^2$	$1.0x10^2$	< 100	20	$1.0x10^3$	$2.3x10^4$	Neg.				
			11:00	$5.0x10^2$	$3.0x10^2$	100	20	$1.0x10^3$	$3.1x10^4$	Neg.	Neg.	11:00 - 11:30	Neg.	
			11:15	$9.0x10^2$	$3.0x10^2$	< 100	20	$3.0x10^3$	$1.4x10^4$	Neg.				
			11:30	$1.8x10^3$	$1.0x10^2$	< 100	30	$3.0x10^3$	$3.6x10^4$	Neg.	Neg.	11:30 - 12:30	Neg.	
			11:45	$4.6x10^3$	$6.0x10^2$	200	10	$1.0x10^3$	$1.7x10^4$	Neg.				
			12:00	$1.4x10^4$	$2.0x10^2$	< 100	70	$4.0x10^3$	$3.0x10^4$	Neg.				
			12:15	$2.3x10^4$	$3.0x10^2$	100	100	$<1.0x10^3$	$6.3x10^4$	Neg.				
			12:30	$1.4x10^4$	$2.0x10^2$	200	90	$1.0x10^3$	$4.2x10^4$	Neg.	Neg.	12:30 - 14:00	Neg.	
			13:00	$6.5x10^3$	$5.0x10^2$	100	80	$1.0x10^3$	$6.5x10^4$	Neg.				
			13:30	$2.9x10^3$	< 100	100	80	$2.0x10^3$	$1.3x10^4$	Neg.				
			14:00	$1.8x10^3$	$1.0x10^2$	< 100	20	$2.0x10^3$	$2.2x10^4$	Neg.				

TABLE A1 (c). MICROBIOLOGICAL DATA FROM MALVERN ROAD STORM SEWER, BURLINGTON, APRIL 21 - OCTOBER 24, 1976.

Date	Storm		Time of Sample Collection	Coliforms /100 ml	Fecal Coliforms /100 ml	Fecal Strepto-cocci /100 ml	P. aeru-ginosa /100 ml	Fungi /100 ml	Hetero-troph Count /ml	Salmonella /100 ml	Lepto-spira /100 ml	Time of Swab Collection	Salmonella Collecting Swab	Virus Collecting Swab
	Duration	Amount Rainfall												
July 7	150 min.	7 mm	18:15	2.6×10^{2}	40	90	20	5.0×10^{2}	4.8×10^{4}	Neg.	Neg.	18:15-	S. thompson	Neg.
			18:25	2.2×10^{4}	2.5×10^{3}	2.0×10^{4}	3.7×10^{3}	2.1×10^{5}	8.4×10^{5}	S. thompson	Neg.	18:45		
			18:35	2.3×10^{4}	8.3×10^{2}	6.2×10^{4}	1.1×10^{3}	2.9×10^{5}	7.3×10^{5}	Neg.				
			18:45	8.2×10^{3}	3.2×10^{3}	1.4×10^{4}	2.0×10^{3}	2.8×10^{5}	4.0×10^{5}	Neg.	Neg.	18:45-	Neg.	Neg.
			19:00	9.3×10^{3}	1.3×10^{3}	2.0×10^{4}	3.0×10^{3}	2.7×10^{5}	2.6×10^{5}	Neg.		19:15		
			19:15	8.5×10^{3}	1.4×10^{3}	1.3×10^{4}	2.7×10^{3}	1.6×10^{5}	2.7×10^{5}	Neg.	Neg.	19:15-	Neg.	Neg.
			19:30	5.6×10^{3}	1.2×10^{3}	1.3×10^{4}	3.5×10^{3}	1.2×10^{5}	3.2×10^{5}	Neg.		20:15		
			19:45	6.9×10^{3}	1.8×10^{3}	1.4×10^{4}	3.5×10^{3}	1.0×10^{5}	3.4×10^{5}	Neg.				
			20:00	5.2×10^{3}	9.0×10^{2}	1.0×10^{4}	3.5×10^{3}	9.8×10^{4}	3.1×10^{5}	Neg.				
			20:15	5.3×10^{3}	1.5×10^{3}	1.0×10^{4}	3.2×10^{3}	8.0×10^{4}	5.2×10^{5}	Neg.	Neg.	20:15-	Neg.	Neg.
			20:45	3.7×10^{3}	7.0×10^{2}	5.3×10^{3}	2.3×10^{3}	5.3×10^{4}	7.0×10^{5}	Neg.		20:45		
Aug. 5	240 min.	9 mm	16:25	1.2×10^{4}	2.0×10^{3}	8.0×10^{3}	130	1.0×10^{2}	2.8×10^{5}	Neg.	Neg.	16:25-	Neg.	Neg.
			16:35	1.5×10^{4}	1.1×10^{3}	1.9×10^{4}	130	2.0×10^{3}	3.8×10^{5}	Neg.	Neg.	16:55		
			16:45	8.5×10^{3}	8.0×10^{2}	1.8×10^{4}	130	< 100	2.6×10^{5}	Neg.				
			16:55	6.3×10^{3}	7.0×10^{2}	6.9×10^{3}	450	7.0×10^{3}	9.6×10^{4}	Neg.	Neg.	16:55-	Neg.	Neg.
			17:10	2.0×10^{3}	2.8×10^{2}	3.0×10^{2}	70	8.0×10^{3}	6.4×10^{4}	Neg.		17:25		
			17:25	7.1×10^{3}	8.8×10^{2}	1.4×10^{4}	320	1.4×10^{4}	4.0×10^{5}	Neg.	Neg.	17:25-	Neg.	Neg.
			17:40	2.2×10^{3}	3.2×10^{2}	2.0×10^{4}	280	7.0×10^{3}	3.0×10^{5}	Neg.		18:25		
			17:55	4.0×10^{3}	4.1×10^{2}	2.9×10^{4}	420	8.0×10^{3}	3.6×10^{5}	Neg.				
			18:10	3.3×10^{3}	4.7×10^{2}	1.7×10^{4}	430	8.0×10^{3}	4.0×10^{5}	Neg.				
			18:25	2.5×10^{3}	3.4×10^{2}	5.0×10^{3}	900	4.0×10^{3}	1.2×10^{5}	Neg.	Neg.	18:25-	Neg.	Neg.
			18:55	3.0×10^{3}	2.2×10^{2}	5.8×10^{3}	200	2.7×10^{3}	6.6×10^{5}	Neg.		20:25		
			19:25	4.8×10^{3}	3.0×10^{2}	4.6×10^{3}	100	1.2×10^{3}	1.5×10^{5}	Neg.				
			20:25	1.6×10^{3}	1.1×10^{2}	8.0×10^{2}	280	1.3×10^{3}	3.2×10^{5}	Neg.				

TABLE A1 (d). MICROBIOLOGICAL DATA FROM MALVERN ROAD STORM SEWER, BURLINGTON, APRIL 21 - OCTOBER 24, 1976.

Date	Storm		Time of Sample Collection	Coliforms /100 ml	Fecal Coliforms /100 ml	Fecal Strepto-cocci /100 ml	P. aeru-ginosa /100 ml	Fungi /100 ml	Hetero-troph Count /ml	Salmonella /100 ml	Lepto-spira /100 ml	Time of Swab Collection	Salmonella Collecting Swab	Virus Collecting Swab
	Duration	Amount Rainfall												
Aug. 13	240 min.	5 mm	07:40	3.4×10^{2}	1.3×10^{2}	< 100	10	1.0×10^{2}	3.0×10^{4}	Neg.	Neg.	07:40-	Neg.	
			07:50	7.0×10^{2}	60	6.0×10^{2}	< 10	< 100	6.0×10^{4}	Neg.	Neg.	08:10		
			08:00	1.0×10^{2}	90	4.0×10^{2}	10	4.0×10^{2}	1.0×10^{4}	Neg.				
			08:10	1.1×10^{3}	1.4×10^{2}	7.0×10^{2}	20	7.0×10^{2}	< 1000	Neg.	Neg.	08:10-	Neg.	
			08:25	< 100	< 10	< 100	20	5.0×10^{2}	7.0×10^{4}	Neg.		08:40		
			08:40	3.0×10^{2}	4.0×10^{2}	3.6×10^{3}	3.6×10^{2}	8.0×10^{3}	2.4×10^{5}	Neg.	Neg.	08:40-	Neg.	
			08:55	1.1×10^{3}	4.7×10^{3}	2.2×10^{3}	9.0×10^{2}	6.0×10^{3}	1.4×10^{5}	Neg.		09:40		
			09:10	2.9×10^{3}	1.8×10^{3}	1.3×10^{3}	2.0×10^{3}	8.0×10^{3}	2.4×10^{5}	Neg.				
			09:25	4.8×10^{4}	8.0×10^{2}	4.9×10^{3}	5.0×10^{2}	5.0×10^{3}	2.0×10^{5}	Neg.				
			09:40	2.2×10^{4}	1.6×10^{3}	4.6×10^{3}	7.0×10^{2}	9.0×10^{3}	1.4×10^{5}	Neg.	Neg.	09:40-	Neg.	
			10:10	1.7×10^{4}	1.5×10^{3}	4.5×10^{3}	8.0×10^{2}	1.6×10^{3}	8.0×10^{4}	Neg.		11:40		
			10:40	1.2×10^{4}	1.6×10^{3}	5.8×10^{3}	8.0×10^{2}	9.0×10^{2}	9.4×10^{4}	Neg.				
			11:40	4.1×10^{3}	1.3×10^{3}	4.5×10^{3}	1.2×10^{3}	7.0×10^{2}	8.2×10^{4}	Neg.				
Sept. 21	240 min.	10 mm	18:15	1.1×10^{2}	2.0×10^{2}	3.0×10^{2}	10	2.0×10^{2}	4.0×10^{4}	Neg.	Neg.	18:15-	Neg.	Neg.
			18:25	< 100	50	< 100	30	6.0×10^{2}	1.0×10^{5}	Neg.	Neg.	18:45		
			18:35	2.0×10^{2}	80	< 100	50	1.0×10^{2}	1.0×10^{5}	Neg.				
			18:45	1.4×10^{3}	50	6.0×10^{2}	1.1×10^{2}	2.6×10^{4}	2.8×10^{5}	Neg.	Neg.	18:45-	Neg.	Neg.
			19:00	4.7×10^{3}	70	9.0×10^{2}	3.1×10^{2}	3.2×10^{4}	4.4×10^{5}	Neg.		19:15		
			19:15	5.3×10^{3}	1.3×10^{3}	3.2×10^{3}	5.5×10^{2}	3.6×10^{4}	2.4×10^{5}	Neg.	Neg.	19:15-	Neg.	Neg.
			19:30	9.6×10^{3}	2.9×10^{3}	5.3×10^{3}	3.5×10^{2}	2.6×10^{4}	4.4×10^{5}	Neg.		20:15		
			19:45	6.8×10^{3}	3.7×10^{3}	5.0×10^{3}	2.9×10^{2}	2.4×10^{4}	1.3×10^{5}	Neg.				
			20:00	4.8×10^{3}	3.1×10^{3}	4.8×10^{3}	4.2×10^{2}	1.9×10^{4}	1.3×10^{5}	Neg.				
			20:15	3.3×10^{3}	2.8×10^{3}	2.2×10^{3}	3.4×10^{2}	1.2×10^{4}	1.1×10^{5}	Neg.	Neg.	20:15-	Neg.	Neg.
			20:45	5.5×10^{3}	1.2×10^{3}	2.6×10^{3}	2.6×10^{2}	8.0×10^{3}	9.4×10^{4}	Neg.		22:15		
			21:15	4.4×10^{3}	1.0×10^{3}	3.2×10^{3}	2.3×10^{2}	7.0×10^{3}	9.2×10^{4}	Neg.				
			22:15	4.3×10^{3}	4.4×10^{2}	1.2×10^{3}	70	1.5×10^{3}	7.0×10^{4}	Neg.				

TABLE A1 (e). MICROBIOLOGICAL DATA FROM MALVERN ROAD STORM SEWER, BURLINGTON, APRIL 21 - OCTOBER 24, 1976.

Date	Storm		Time of Sample Collection	Coliforms /100 ml	Fecal Coliforms /100 ml	Fecal Strepto-cocci /100 ml	P. aeru-ginosa /100 ml	Fungi /100 ml	Hetero-troph Count /ml	Salmonella /100 ml	Lepto-spira /100 ml	Time of Swab Collection	Salmonella Collecting Swab	Virus Collecting Swab
	Duration	Amount Rainfall												
Oct. 6	240 min.	38 mm	15:00	1.6×10^{3}	5.3×10^{2}	1.0×10^{2}	20	3.0×10^{2}	4.0×10^{4}	Neg.	Neg.	15:00-15:30	Neg.	
			15:10	< 100	< 10	< 100	< 10	1.0×10^{2}	$< 10^{3}$	Neg.				
			15:20	< 100	< 10	< 100	< 10	< 100	$< 10^{3}$	Neg.				
			15:30	< 100	< 10	< 100	< 10	1.0×10^{2}	2.0×10^{4}	Neg.	Neg.	15:30-16:00	Neg.	
			15:45	6.0×10^{2}	1.0×10^{2}	< 100	10	1.3×10^{3}	2.0×10^{4}	Neg.				
			16:00	5.3×10^{4}	2.8×10^{4}	7.4×10^{3}	4.5×10^{2}	1.1×10^{5}	1.8×10^{6}	Neg.	Neg.	16:00-17:00	Neg.	
			16:15	2.7×10^{4}	9.5×10^{3}	3.9×10^{3}	2.4×10^{2}	1.5×10^{5}	2.2×10^{6}	Neg.				
			16:40	9.0×10^{3}	9.3×10^{3}	2.4×10^{3}	3.0×10^{2}	1.2×10^{5}	9.0×10^{5}	Neg.				
			16:45	2.1×10^{4}	1.0×10^{4}	3.7×10^{3}	3.4×10^{2}	1.3×10^{5}	7.2×10^{5}	Neg.				
			17:00	3.5×10^{4}	8.8×10^{3}	5.2×10^{3}	2.6×10^{2}	7.8×10^{4}	5.6×10^{5}	Neg.	Neg.	17:00-19:00	Neg.	
			17:30	2.7×10^{4}	1.2×10^{4}	5.7×10^{3}	2.8×10^{2}	6.4×10^{4}	1.8×10^{5}	Neg.				
			18:00	1.1×10^{4}	9.6×10^{3}	3.6×10^{3}	3.1×10^{2}	4.8×10^{4}	2.8×10^{5}	Neg.				
			19:00	1.1×10^{4}	3.0×10^{3}	3.1×10^{3}	30	1.2×10^{5}	1.6×10^{5}	Neg.				
Oct. 13	75 min.	7 mm	15:50	4.0×10^{2}	1.7×10^{2}	< 100	< 10	< 100	2.0×10^{4}	Neg.		15:50-16:20	Neg.	
			16:00	4.8×10^{3}	1.6×10^{3}	1.0×10^{2}	20	< 100	2.8×10^{4}	Neg.				
			16:10	4.6×10^{4}	1.2×10^{4}	2.0×10^{3}	50	2.2×10^{4}	3.2×10^{5}	Neg.				
			16:20	5.2×10^{3}	1.6×10^{3}	1.0×10^{3}	140	2.9×10^{4}	1.6×10^{5}	Neg.		16:20-16:50	Neg.	
			16:35	9.4×10^{3}	7.6×10^{3}	1.5×10^{3}	80	1.5×10^{4}	1.6×10^{5}	Neg.				
			16:50	1.8×10^{4}	1.1×10^{4}	3.0×10^{3}	100	1.7×10^{4}	1.2×10^{5}	Neg.		16:50-17:05	Neg.	
			17:05	7.0×10^{3}	9.0×10^{3}	9.0×10^{2}	80	1.3×10^{4}	2.4×10^{5}	Neg.				

TABLE A1 (f). MICROBIOLOGICAL DATA FROM MALVERN ROAD STORM SEWER, BURLINGTON, APRIL 21 - OCTOBER 24, 1976.

Date	Storm		Time of Sample Collection	Coliforms /100 ml	Fecal Coliforms /100 ml	Fecal Streptococci /100 ml	P. aeruginosa /100 ml	Fungi /100 ml	Heterotroph Count /ml	Salmonella /100 ml	Leptospira /100 ml	Time of Swab Collection	Salmonella Collecting Swab	Virus Collecting Swab
	Duration	Amount Rainfall												
Oct. 19	240 min.	9 mm	16:40	$6.0x10^2$	$2.0x10^2$	40	< 10	$4.0x10^2$	$1.6x10^4$	Neg.		16:40-	Neg.	
			16:50	$4.0x10^2$	$2.0x10^2$	10	< 10	< 100	$< 10^3$	Neg.		17:10		
			17:00	$1.2x10^3$	< 100	10	< 10	$1.0x10^2$	$8.0x10^3$	Neg.				
			17:10	$8.0x10^2$	$1.0x10^2$	20	< 10	< 100	$2.0x10^4$	Neg.		17:10-	Neg.	
			17:25	$1.8x10^3$	$1.0x10^2$	$1.3x10^2$	< 10	$3.0x10^3$	$3.2x10^4$	Neg.		17:40		
			17:40	$2.2x10^3$	$3.0x10^2$	$4.6x10^2$	10	$7.0x10^3$	$6.4x10^4$	Neg.		17:40-	Neg.	
			17:55	$4.8x10^3$	$1.8x10^3$	$1.3x10^3$	120	$9.0x10^3$	$7.4x10^4$	Neg.		18:40		
			18:10	$8.9x10^3$	$2.1x10^3$	$7.0x10^2$	40	$4.0x10^4$	$2.4x10^5$	S.typhi-murium				
			18:25	$3.2x10^4$	$4.7x10^3$	$4.7x10^3$	40	$6.0x10^3$	$2.6x10^5$	Neg.				
			18:40	$3.1x10^4$	$3.6x10^3$	$4.8x10^3$	70	$3.0x10^3$	$1.8x10^5$	Neg.		18:40-	Neg.	
			19:10	$1.7x10^4$	$4.0x10^3$	$4.7x10^3$	80	$3.0x10^3$	$3.2x10^5$	Neg.		20:40		
			19:40	$2.0x10^4$	$4.6x10^3$	$4.8x10^3$	30	$1.5x10^3$	$2.6x10^5$	Neg.				
			20:40	$2.4x10^4$	$3.3x10^3$	$3.6x10^3$	70	$3.0x10^3$	$1.6x10^5$	Neg.				
Oct. 24	240 min.	16 mm	08:50	$2.8x10^3$	$1.6x10^3$	$2.0x10^3$	< 10	$5.0x10^2$	$6.2x10^4$	Neg.		08:50-	Neg.	
			09:00	$5.1x10^3$	$2.5x10^3$	$5.8x10^3$	20	$1.2x10^4$	$1.0x10^5$	Neg.		09:20		
			09:10	$9.0x10^3$	$3.3x10^3$	$2.5x10^4$	10	$2.0x10^3$	$8.6x10^4$	Neg.				
			09:20	$8.6x10^3$	$5.4x10^3$	$1.8x10^4$	20	$2.6x10^3$	$1.4x10^5$	Neg.		09:20-	Neg.	
			09:35	$8.0x10^3$	$3.2x10^3$	$7.0x10^3$	30	$1.4x10^3$	$3.4x10^5$	Neg.		09:50		
			09:50	$1.3x10^4$	$5.2x10^3$	$2.1x10^4$	50	$2.2x10^3$	$2.6x10^5$	Neg.		09:50-	Neg.	
			10:05	$9.0x10^3$	$3.5x10^3$	$5.2x10^3$	40	$3.7x10^3$	$1.7x10^5$	Neg.		10:50		
			10:20	$1.3x10^4$	$4.0x10^3$	$7.0x10^3$	20	$2.1x10^3$	$2.0x10^5$	S. typhi-murium				
			10:35	$1.0x10^4$	$3.4x10^3$	$7.1x10^3$	30	$1.2x10^3$	$1.2x10^5$	Neg.				
			10:50	$6.0x10^3$	$3.8x10^3$	$3.2x10^3$	20	$1.5x10^3$	$2.0x10^5$	Neg.		10:50-	Neg.	
			11:20	$9.1x10^3$	$3.2x10^3$	$3.6x10^3$	40	$1.1x10^3$	$1.8x10^5$	Neg.		12:50		
			11:50	$4.7x10^3$	$3.3x10^3$	$2.2x10^3$	10	$6.0x10^2$	$1.0x10^5$	Neg.				
			12:50	$6.2x10^3$	$2.7x10^3$	$4.0x10^2$	<10	$6.0x10^2$	$1.2x10^5$	S. typhi-murium				

APPENDIX TABLE A2. SUMMARY OF COLIFORM AND FECAL COLIFORM BIOTYPES ISOLATED FROM MALVERN ROAD STORM SEWER, STORM SAMPLES, 1976.

Species or Genera	0-30 Minutes				60-90 Minutes				120-240 Minutes			
	Coliform		Fecal Coliform		Coliform		Fecal Coliform		Coliform		Fecal Coliform	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Storm #6(5/8/76)												
E. coli	1	6	25	100	4	21	25	100	5	23	25	100
Klebsiella	13	72			6	32			10	45		
Enterobacter	2	11			4	21			6	27		
Citrobacter	2	11			5	26			1	5		
Total	18	100	25	100	19	100	25	100	22	100	25	100
Storm #7(13/8/76)												
E. coli	13	52	25	100	2	8	25	100	6	27	25	100
Klebsiella	3	12			17	68			13	59		
Enterobacter	7	28			4	16			3	14		
Citrobacter	2	8			2	8			0	0		
Total	25	100	25	100	25	100	25	100	22	100	25	100
Storm #9(21/9/76)												
E. coli	7	35	23	92	15	65	25	100	6	25	25	100
Klebsiella	2	10	2	8	1	4			8	33		
Enterobacter	8	40			5	22			7	29		
Citrobacter	3	15			2	9			3	13		
Total	20	100	25	100	23	100	25	100	24	100	25	100
Storm #11(19/10/76)												
E. coli	16	64	25	100	16	67	25	100	17	68	25	100
Klebsiella	3	12			7	29			1	4		
Enterobacter	4	16			1	4			4	16		
Citrobacter	2	8			0	0			3	12		
Total	25	100	25	100	24	100	25	100	25	100	25	100
Storm #12(24/10/76)												
E. coli	13	54	25	100	13	57	24	100	20	80	25	100
Klebsiella	5	21			5	22			2	8		
Enterobacter	4	17			4	17			2	8		
Citrobacter	2	8			1	4			1	4		
Total	24	100	25	100	23	100	24	100	25	100	25	100

TABLE A3. SUMMARY OF COLIFORM AND FECAL COLIFORM BIOTYPES ISOLATED FROM MALVERN ROAD STORM SEWER, INFILTRATION SAMPLES, 1976.

Species or Genera	Coliform			Fecal Coliform		
	No.	%	%EC+	No.	%	%EC+
Sample #4(20/7/76)						
E. coli	18	72	78	25	100	92
Klebsiella	4	16	0			
Enterobacter	3	12	0			
Citrobacter	0	0	0			
Total	25	100		25	100	
Sample #6(31/8/76)						
E. coli	16	67	88	25	100	96
Klebsiella	3	13	75			
Enterobacter	4	16	25			
Citrobacter	1	4	0			
Total	24	100		25	100	
Sample #7(29/9/76)						
E. coli	24	96	71	25	100	92
Klebsiella	1	4	0			
Enterobacter	0	0	0			
Citrobacter	0	0	0			
Total	25	100		25	100	
Sample #9(2/11/76)						
E. coli	13	52	100	25	100	88
Klebsiella	9	36	0			
Enterobacter	2	8	0			
Citrobacter	1	4	0			
Total	25	100		25	100	
Sample #10(30/11/76)						
E. coli	3	12	0	25	100	100
Klebsiella	19	76	27			
Enterobacter	3	12	67			
Citrobacter	0	0	0			
Total	25	100		25	100	

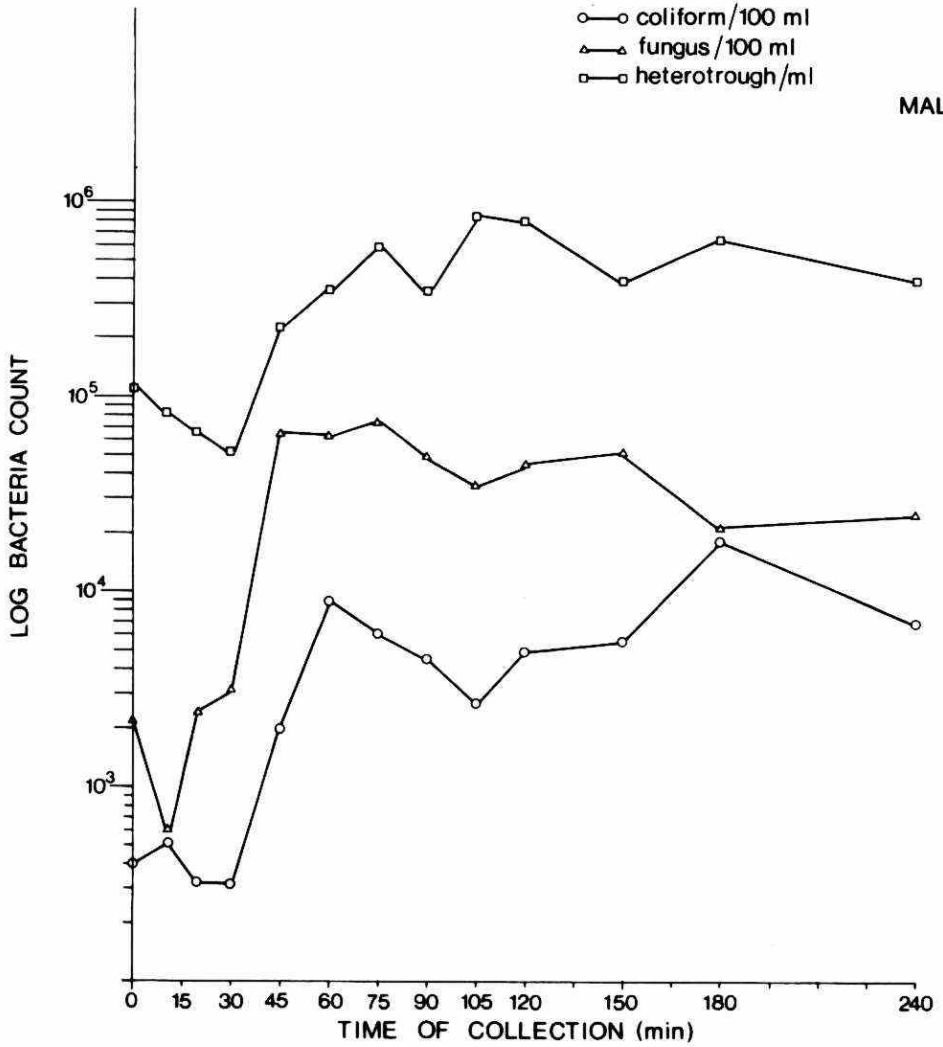
APPENDIX
FIG. 1

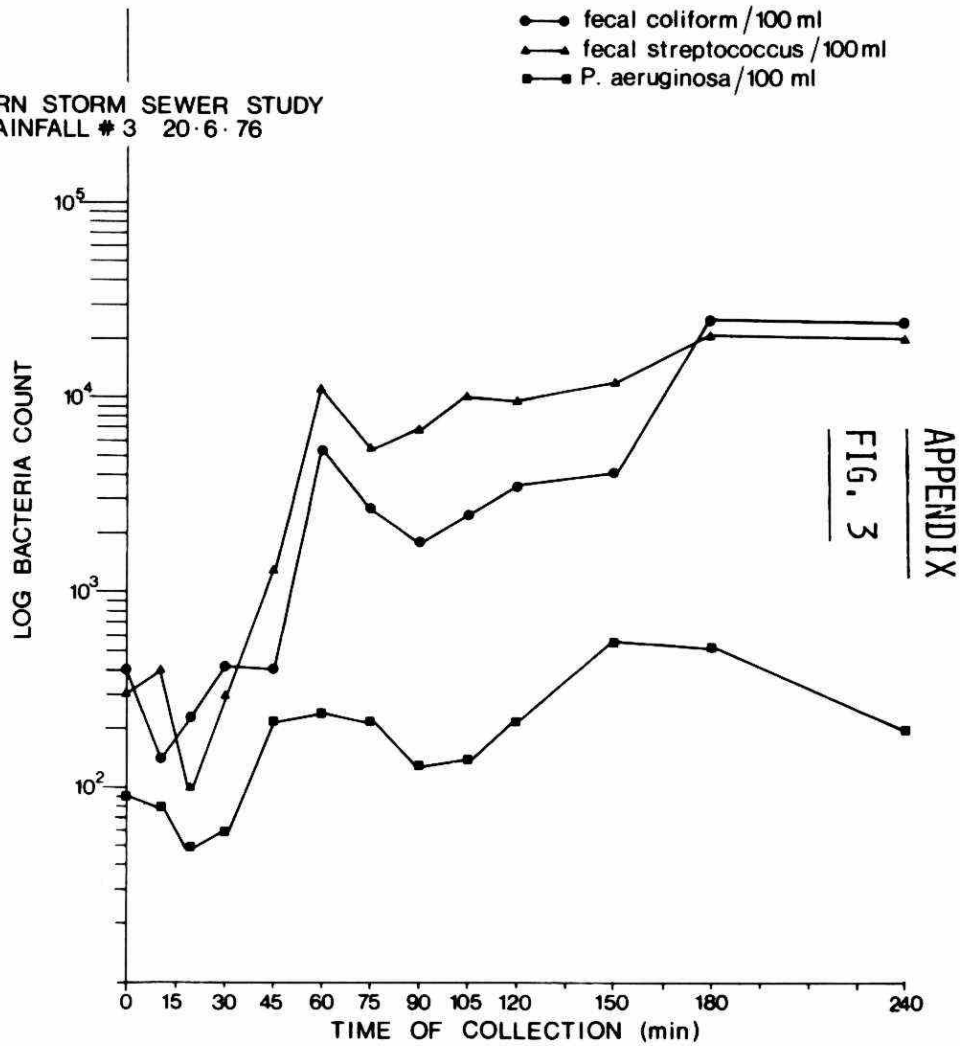
MALVERN STORM SEWER STUDY
RAINFALL #1 21·4·76

APPENDIX

FIG. 2

MALVERN STORM SEWER STUDY
RAINFALL # 2 30·5·76





APPENDIX
FIG. 3

APPENDIX

FIG. 4

MALVERN STORM SEWER STUDY
RAINFALL #4 24·6·76

APPENDIX

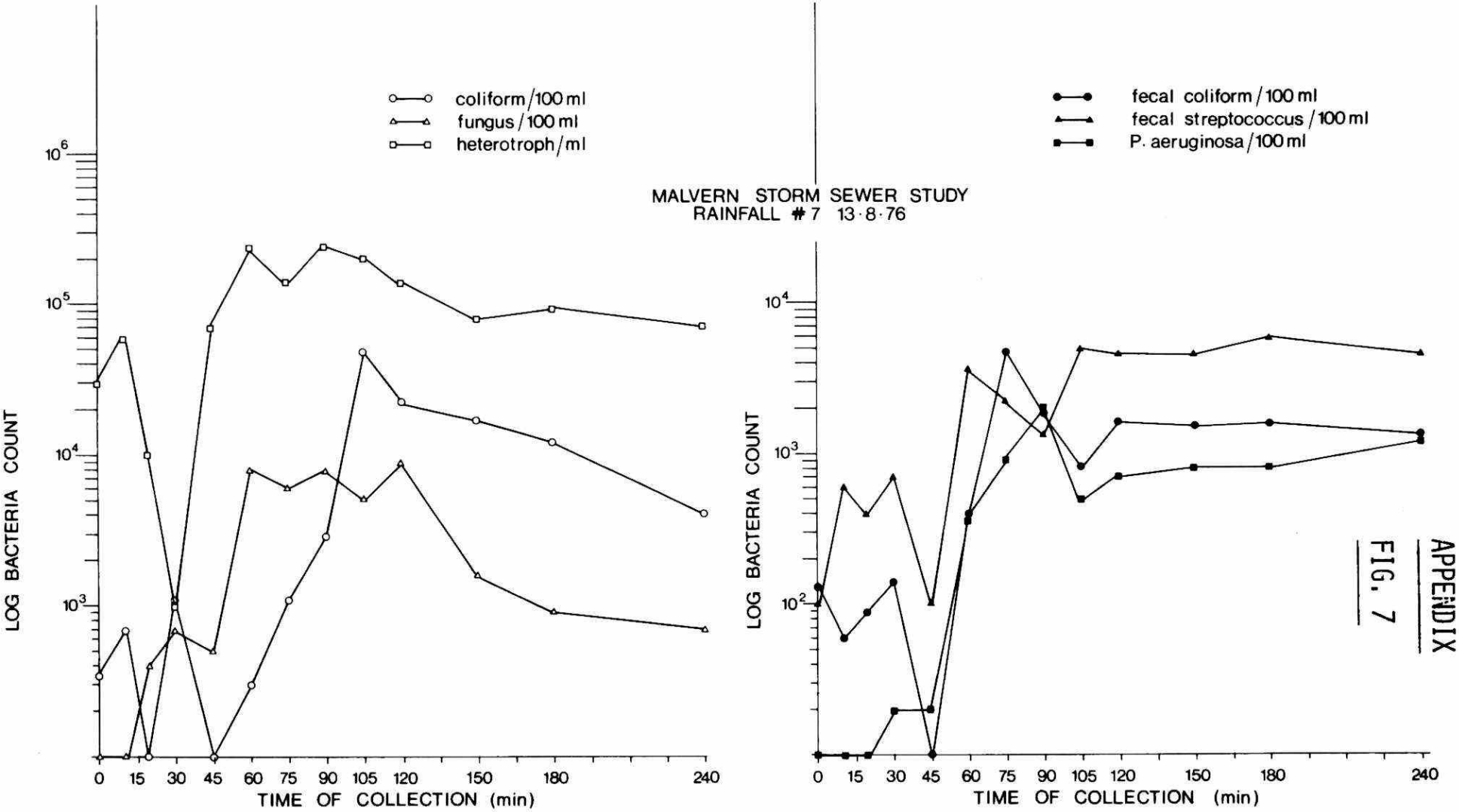
FIG. 5

MALVERN STORM SEWER STUDY
RAINFALL #5 7-6-76

APPENDIX

FIG. 6

MALVERN STORM SEWER STUDY
RAINFALL # 6 5·8·76



APPENDIX
FIG. 7

APPENDIX
FIG. 8

MALVERN STORM SEWER STUDY
RAINFALL # 8 21·9·76

APPENDIX
FIG. 9

MALVERN STORM SEWER STUDY
RAINFALL # 10 13·10·76

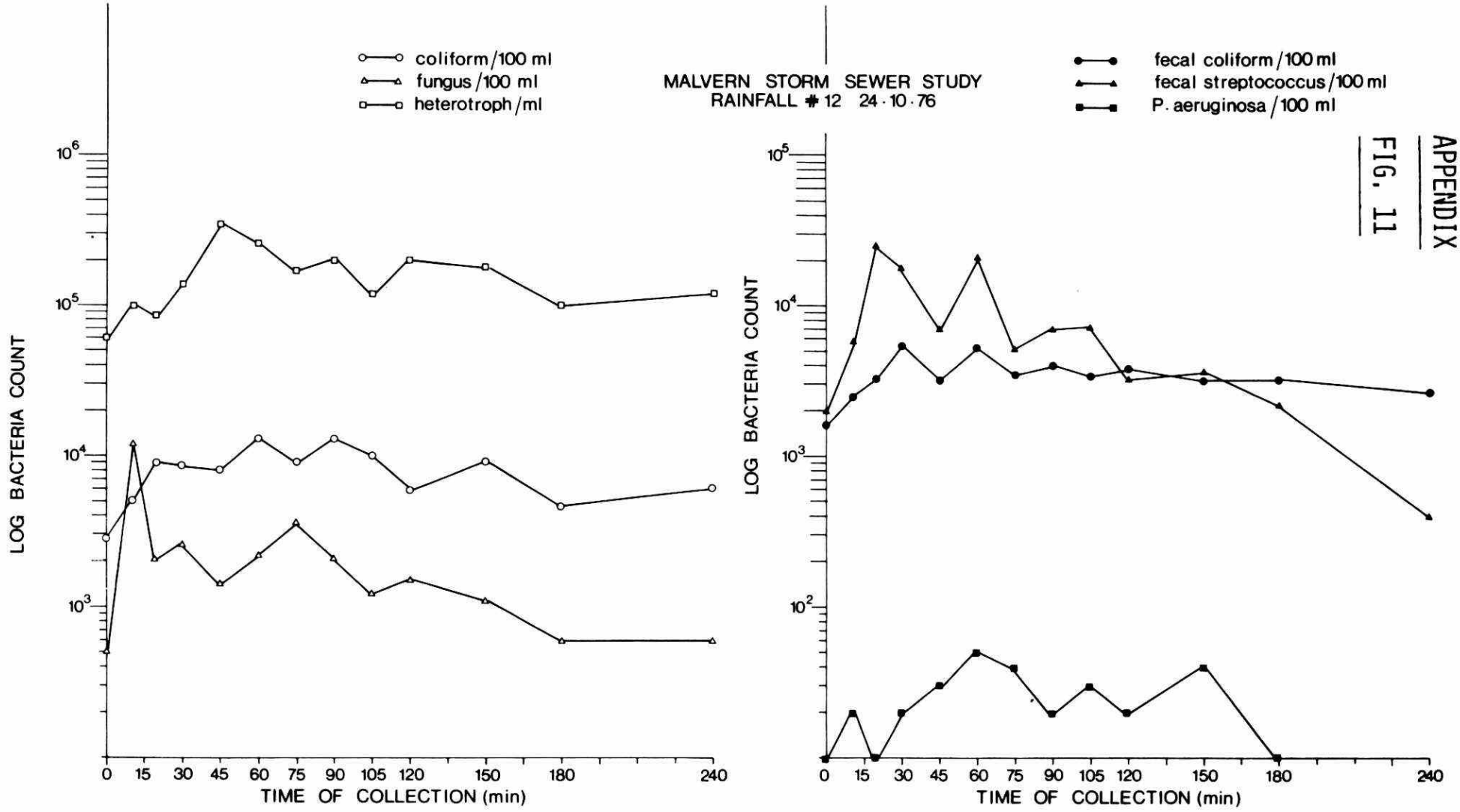
APPENDIX

FIG. 10

MALVERN STORM SEWER STUDY
RAINFALL # 11 19·10·76

APPENDIX

FIG. 11



APPENDIX

FIG. 12

EFFECT OF MALVERN STORM SEWER RUNOFF ON DOWNSTREAM CREEK. MEAN DATA, 12 RAINSTORMS.

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Microbiological characteristics of urban storm water runoffs in Central Ontario.

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